FINAL TECHNICAL REPORT

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This Report is submitted in fulfillment of the terms of the Research Agreement
between
The University of Toronto
and the
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Summary

The subcontract covered a period of fifteen months (Oct/98 to Jan/99) and the objectives were several fold (see pg 5), but the emphasis was on work with the so-called "adapted" variant of recombinant *Zymomonas* 39676:pZB4L. This strain was isolated at NREL from a long-term (5 month) continuous fermentation operating at pH 5.75 and a dilution rate of 0.03/h in which the amount of detoxified (overlimed) hardwood (yellow poplar) dilute-acid prehydrolysate was increased incrementally to a level of 50% (0.75% w/v acetic acid). The level of xylose and glucose was kept constant at 4% (w/v) and 0.8%, respectively; these sugar concentrations reflect the composition of the undiluted hardwood prehydrolysate (Lawford *et al.*, Appl. Biochem. Biotechnol. 70-72, 353, 1998).

The work performed as part of this subcontract has been described previously in the context of the following:

(i) 8 Technical Progress Reports (Sept/98 - Dec/98)

(ii) Papers #75 and #76 - *20th Symposium on Biotechnology* (May, 1998) {Published in *Appl. Biochem. Biotechnol.* 77-79, 1999}

(iii) Seminar presented at NREL, March 2, 1998

In addition to the above, the continuous fermentation work with the adapted strain is the subject of a paper being presented at the *21st Symposium on Biotechnology* (May, 1999)

The objectives for this work are listed at the end of this Summary (see pg 5). The work involved pH-controlled batch, fed-batch and continuous fermentations using bench-scale bioreactors. Although the focus was on the "adapted" strain, direct comparisons were made in side-by-side fermentations to other NREL recombinant Zm strains, including CP4:pZB5 and the non-adapted 39676:pZB4L. In the absence of dilute-acid hydrolysate, pure sugar synthetic prehydrolysate media were used to assess growth and fermentation performance as a function of (i) nutrient composition of the medium, (ii) the ratio of sugars (glucose to xylose), (iii) the effect of acetic acid, and (iv) the effect of ethanol. The fact that prehydrolysate was not used in this work is viewed by us as a major limitation to strain characterization and one which significantly compromises any conclusions regarding possible strain superiority in terms of commercial potential since the "real" test could not be performed.

In batch fermentations with a nutrient-rich yeast extract-based medium and glucose as the sole sugar, both the cell mass concentration and the rate of glucose utilization were slower with the adapted strain compared to the other recombinants. With xylose as sole sugar, growth and fermentation were slower with the adapted variant compared to the other strains. Fermentation performance by all three recombinants was very similar in the synthetic prehydrolysate medium

containing 4% xylose and 0.8% glucose (without acetic acid) - the batch fermentation being complete in about 24h with a sugar-to-ethanol conversion efficiency of 95%. In media containing acetic acid (range 0.2-1% w/v) at pH 5.75-6.0, the rate of xylose utilization is considerably faster with CP4:pZB5 than 39676:pZB4L. The adapted strain exhibited a rate which is intermediate between the other recombinants. Growth and fermentation were improved in acetic acid media when higher "pitching" was employed (ie. use of larger inoculum).

In previous work, the fermentation media routinely contained a ratio of xylose to glucose of 5:1 which is a characteristic of NREL's dilute-acid hardwood prehydrolysate - the total sugar concentration being 4.8% (w/v). However, not all feedstocks have the same sugar composition and this study revealed the importance of the sugar ratio with respect to the rate of xylose conversion. There are several factors which impact on the sugar ratio effect. Although both sugars can be used simultaneously by recombinant *Zymomonas*, because of the nature of the sugar transport system, there is an apparent preference for glucose over xylose. Furthermore, the transport characteristics of the adapted strain appear to differ from the other recombinants. This study showed that at higher levels of glucose relative to xylose, xylose utilization is delayed. However, because the growth yield from glucose is higher than from xylose, an increased amount of glucose results in a higher cell density and this counters the competitive transport effect. Also growth on glucose is less inhibited by acetic acid than growth on xylose. At higher sugar loadings (ca 10-12%), ethanol can be a factor since xylose conversion is more sensitive to ethanol inhibition. This work showed that, in batch fermentations at high sugar loading, xylose utilization was halted at ethanol concentrations in the range 55-60 g/L. In fermentations where the sugar loading was about 5% (w/v), the rate of xylose conversion was significantly slower at xylose to glucose mass ratio <3:1. This has practical implications in terms of feedstocks such as corn stover hydrolysate where the xylose to glucose ratio is inversed or about 0.3:1

This work extended previous studies on the capacity of corn steep liquor (CSL) to satisfy the nutritional requirements of recombinant *Zymomonas*. To facilitate turbidity measurements, this study employed 1% (v/v) clarified CSL, but in term of N content, whole slurry CSL is nutritionally equivalent on a mass basis. This level of CSL is adequate for robust growth and cofermentation by rec Zm at sugar loading of about 5%. The nutritionally effectiveness of whole CSL (increasing available N) can be improved by hydrolyzing the protein. The cost of CSL is a driver for seeking either a reduction in the amount used or an alternative cost-effective nutrient source. Both the adapted and CP4:pZB5 recombinants performed better than the 39676:pZB4L in a medium containing 0.25% cCSL + 0.12% DAP and 0.4% acetic acid (pH 5.75). Although tap water can provide many of the important inorganic elements, magnesium supplementation appears to be important. At NREL's current cost for CSL of 6¢/lb, the cost of nutrients using only whole CSL at 1% (w/v) would be about 8¢/gal ethanol. This cost would be reduced to about 6.6¢/gal for 0.25% CSL where DAP and Mg were also added.

In continuous fermentations with the adapted strain, media with 1% cCSL produced almost identical fermentation performance relative to the bench-mark nutrient-rich YE-based medium. At the sugare loadings used (4.8% total sugars) 1% CSL seemed to satisfy the nutritional requirement with the cell mass being less than 2 gDCM/L. The CSL-based media were prepared using distilled water and a complete salts supplement ("Z salts") could be replaced by magnesium (range 1.5-2mM). However, this level of Mg supplementation would not be necessary in an industrial setting where non-deionized water would be used. The adapted strain performed equally well in media containing either 4% xylose + 0.8% glucose or 3% xylose + 1.8% glucose. With these two media, the pattern of steady-state cell mass and effluent xylose concentrations as a function of dilution rate were virtually superimposable. The maximum growth yield was about 0.032 gDCM/ g sugars and the maintenance energy coefficient (m) was 0.11 and 0.4 g sugar/g cell/h in the standard nutrient-rich medium and 1% CSL medium, respectively. The apparent uncoupling effect of CSL (ie. increased m value) has been observed in previous work. Under these conditions the adapted strain behaved in a similar fashion to the parent strain 39676:pZB4L (Fig. D-20, Final Report, 1997). However, these values for both growth yield and maintenance energy differ somewhat from those quoted for a continuous fermentation conducted at NREL with the parent strain 39676:pZB4L using a 1%cCSL medium and 4% xylose + 0.8% glucose (19th Symposium paper published in ABAB, 70-72, 353-367). All these are within the range of values for *Zymomonas* in the literature.

At a constant dilution rate of 0.04/h (ie. residence time of about 1 day) the volumetric productivity for 4% X + 0.8% G and the 3%X + 1.8% G CSL-based media was 0.78 and 0.82 g ethanol/L/h, respectively. When 0.4% (w/v) acetic acid was added to these two different feed media, the productivity decreased slightly to 0.67 and 0.74 g ethanol/L/h, respectively. With 0.4% acetic acid at pH 5.75, there was about 10-15% reduction in productivity and the level of effluent xylose was independent of the sugar ratio of the medium. The presence of 0.4% acetic acid at pH 5.75 did not alter the near theoretical metabolic yield 0.48 g ethanol/g sugars used (94%); however, the process conversion efficiency was only 80% due to the elevated level of unfermented xylose.

In the case of the medium containing 4% xylose + 0.8% glucose. 0.4% acetic acid at the permissible pH of 5.75 caused the growth yield to decrease to 0.023 while the m value was increased only slightly to 0.54. In the case of the medium containing 3% xylose + 1.8% glucose, the same amount of acetic acid (0.4% at pH 5.75) did not alter the growth yield; however, the m value was doubled from 0.46 to 1.0 g sugar/ g cell/h

Using a 1% cCSL-based medium containing 3% xylose and 1.8% glucose at pH 5.75, the addition of 1.2% w/v) ethanol to the feed did not alter the continuous fermentation performance of the adapted strain in terms of the level of exit xylose as a function of D; however, the combination 0.2% acetic acid and 1.2% ethanol resulted in much higher effluent xylose levels at d values 0.04/h -to 0.06/h. It appears that ethanol exacerbates the inhibitory effect of acetic acid at the levels and conditions tested.

A factor in this work which mitigates very strongly against the certainty or validity of any conclusions regarding either strain performance superiority or the effects produced by alterations to media composition (eg sugar ratio or amount of potential inhibitory substances) as these relate to the stated objectives is the persistent lack of reproducibility of repeat experiments especially at higher sugar loadings >4.8% (w/v). Examples of experimental variation in batch fermentations are highlighted in Table 6. One possible explanation for the observed deviations is strain variation and the present procedures used for stock culture maintenance can not rule out this possibility. Time did not permit an analysis of this phenomenon of apparent non-reproducibity but the physiological state of the inoculum was identified as being important. In continuous fermentations, the start up is an important period and the time that flow is initiated relative to the level of residual xylose, appears to be critical. In repeat experiments, very different patterns with respect to effluent xylose were observed. The cell density of the batch culture prior to starting the continuous fermentation is another factor that was identified as being important and the amount of growth promoting glucose appears to play a key role here.

All continuous fermentations involved antibiotic in the medium for maintenance of the plasmid-bearing recombinant and consequently to ensure xylose fermentation. However, the use of antibiotics will not likely be permissible in the context of large-scale operations for several reasons. Therefore, NREL's recent announcement heralding the advent of the chromosomally integrated construct is particularly timely. Future work will need to ensure that this new entry into the competition for a place in the commercial production of fuel ethanol from biomass meets the performance criteria already established by its predecessors.

Finally, the continuous fermentation work with the adapted strain will be the subject of a presentation at the upcoming *21st Symposium on Biotechnology for Fuels and Chemicals* (Fort Collins, CO - May 2-6, 1999). This paper which will be submitted to Humana Press for publication in *Applied Biochemistry and Biotechnology* will detail aspects of this work that are not covered in this Report. Furthermore, a second paper is contemplated that will deal with interesting comparative aspects of the bioenergetics of xylose and glucose metabolism by recombinant Zm CP4:pZB5 (Abstract submitted to 21st Symposium).

Objectives as per the s/c SOW are listed on following page

OBJECTIVES

(1) *SOW Task 2.
To verify batch fermentation performance of NREL's "adapted" rZ 39676pZB4L strain under standard conditions and to confirm the sufficiency of 2%(v/v) cCSL to support effective fermentation performance.

(2) Extension Task 3
To conduct batch fermentations to examine the effect of glucose on the rate of xylose utilization for concentrations of xylose in the range 4%-8%. These experiments will include the use of acetic acid containing media over the pH 5 to pH 6 range.

(3) Extension Task 4
To conduct batch fermentations to examine the requirement for medium supplementation (requirement for DAP and/or Mg) with reduced level of CSL.

(4) SOW Task 3
To characterize performance in continuous culture of various feed sugar ratios as a function of dilution rate over the operating range of 0.04 - 0.10 h-1.

(5) SOW Task 4
To assess the effect of acetic acid on chemostat performance using two feed sugar and two acetic acid concentrations.

(6) SOW Task 5
To assess the effect of ethanol on chemostat performance using two feed sugar ratios and to assess the effect of ethanol on media containing 0.2%(w/v) acetic acid.

*SOW Task 1 = QA/QC

{as per Statement of Work - 09/29/97 and modified Statement of Work(extension) - 07/23/98}

MATERIALS AND METHODS

Organisms

The recombinant *Zymomonas mobilis* (strain ATCC 39676 carrying the plasmid pZB4L) (Zhang *et al.*,1995) and the adapted strain *Z. mobilis* ATCC 39676pZB4L were received from A. Mohagheghi (NREL, Golden, CO, USA) in Nov, 1997.

Long-term storage/maintenance of organism

Plasmid-bearing cultures, grown from single colony isolates on selective agar medium (xylose + tetracycline), were stored at -70°C in RM medium supplemented with antifreeze (glycerol at 15ml/dl). The phenotypic characteristics of the recombinant culture were related to growth in the presence of tetracycline and the production of ethanol from D-xylose. Cultures were generally revived in RM medium that contained 2% (w/v) glucose and 2% (w/v) xylose .

Fermentation equipment

Batch fermentations were conducted in 2L MultiGen stirred-tank bioreactors (Model F2000, New Brunswick Scientific, Edison, NJ) fitted with agitation (100 RPM), pH, and temperature control (30°C). The working volume was 1500 ml and the pH was controlled by the addition 4N KOH (NBS model pH-40 controller). Continuous fermentations were conducted with 750mL MultiGen bioreactors (Model F1000, New Brunswick Scientific, Edison, NJ) or 2L BioFlo bioreactors (Model BioFlo 2000, New Brunswick Scientific, Edison, NJ). The working volume of the chemostats was about 350 mL or 1500mL. Steady-state was assumed only after a minimum of three volumes had exchanged and when samples taken on successive days gave similar values with substrate and product concentrations. In all fermentations the amount of pre-culture ('inoculum') added was sufficient to produce an OD_{600nm} reading (1 cm light path) of 0.2-0.25 (equivalent to an initial cell density of approximately 60-75 mg dry cell mass/L).

Methods of pre-culture and inoculation procedures

A 1ml aliquot of a glycerol preserved culture was removed from cold storage (freezer) and transferred to about 100 ml of complex medium (RM), containing about 2%(w/v) xylose and 2% (w/v) glucose supplemented with tetracycline (Tc) (10mg/L), in 125 ml screw-cap flasks and grown statically overnight at 30°C in an incubator (GCA/Precision Scientific Group,Model 4EG). This pre-seed culture was transferred to inocula flasks containing RM, 2% glucose & 2% xylose and Tc and were also grown statically overnight at 30°C in an incubator. The inocula flasks were used at a level of ~10%(v/v) to inoculate the fermentations.

Fermentation media

The composition of the different media used in this study are described in Table 1. Bacto Yeast Extract (YE) and Bacto Tryptone were obtained from Difco Laboratories (Detroit, MI). Other chemicals were laboratory-grade purity. For comparative purposes, the nutrient-rich, complex culture medium described by Goodman *et al.* (1982) was used as the "bench mark" or reference standard. This medium is commonly referred to as "RM" (Table 1). A medium containing less YE, supplemented with Zymo salts, designated as "ZM" was also used (Table 1). The corn steep liquor (CSL) medium consisted of autoclaved tap water (TW) or distilled H_2O supplemented with corn steep liquor (cCSL) (diluted 1:4 with distilled H_2O and then centrifugally clarified) which was added at the time of inoculation. This procedure was adopted because it was observed that autoclaving the CSL resulted in a precipitate forming which interfered in accurate cell mass determinations. Consequently it was decided to add non-sterile cCSL at the beginning of fermentation. Because of the relative short term nature of these batch fermentations and the rate of inoculation, problems caused by contamination were not anticipated to occur. For longer-term chemostat operations, the CSL media were autoclaved.

A synthetic "biomass prehydrolyzate" (BPH) was formulated to model the composition of the NREL hardwood dilute-acid prehydrolyzate (Table 1). The synthetic BPH medium was made with distilled water and contained 4% (w/v) xylose, 0.8% (w/v) glucose and 0 - 0.75% (w/v) acetic acid; it was nutritionally supplemented with either RM components or CSL. All media contained 10mg/L tetracycline. All media were sterilised by autoclaving at 121°C for 30-45 minutes. Stock sugar solutions were autoclaved separately. Tetracycline was added to the sterilised medium after cooling.

Analytical procedures

Growth was measured turbidometrically at 600nm (1cm lightpath) (Unicam spectrophotometer, model SP1800). In all cases the blank cuvette contained distilled water. Dry cell mass (DCM - often referred to by microbial physiologists as "biomass") was determined by microfiltration of an aliquot of culture followed by washing and drying of the filter to constant weight under an infrared heat lamp. It was found that 1 OD corresponded (on average) to 0.3 gDCM/L. Compositional analyses of fermentation media and cell-free spent media was accomplished by HPLC with a RI monitor and computer-interfaced controller/integrator (Bio-Rad Labs, Richmond, CA). Separations were performed at 65°C using an HPX-87H column (300 x 7.8mm) (Bio-Rad Labs, Richmond, CA). The mobile phase was 0.005M sulphuric acid (flow rate = 0.6ml/min.) and the injection volume was 0.02 ml. Standards for D-xylose, DL-lithium lactate, and potassium acetate were prepared from research grade chemicals using a micro balance and diluting with distilled water using a volumetric flask. D-glucose and ethanol standards were purchased from Sigma (Sigma Chemical Co., St.Louis, MO) The xylose standard was 2% (w/v) and all others were about 1% (w/v). These standards were run on a routine basis (ie. for each batch of samples analysed).

Table 1 *Zymomonas* media formulations

Ingredient (g)	Medium Designation				
	RM •	ZM1 *	ZM	cCSL	Lo cCSL
Yeast Extract (Difco)	10.0	3.0	5.0		
cCSL (mL)				50	12.5
NH_4Cl	-	0.8	0.8	-	-
$(NH_4)_2HPO_4$ (DAP)	-	-	-	-	1.23
KH_2PO_4	2.0	3.48	3.48	3.48	3.48
$MgSO_4.7H_2O$	-	1.0	1.0	1.0	1.0
$FeSO_4.7H_2O$	-	0.01	0.01	0.01	0.01
Citric acid	-	0.21	0.21	0.21	0.21
Distilled water (L)	1	1	1	1	1

Clarified CSL (cCSL) = CSL diluted 1:4, centrifuged and filter sterilzed

• Goodman *et al.* (1982) *Appl. Environ. Microbiol.*, 44(2): 496-498

* Lawford (1988) *Appl. Biochem. Biotechnol.*, 17: 203-209

Analysis for nitrogen content of CSL and cell mass

The corn steep liquor used in this work was supplied by NREL (GPC,Muscatine,IA). Assays for total nitrogen (Kjeldahl) were performed on samples of whole and clarified CSL (Table 2) and the cell mass of the recombinant (MicroChem Labs, Toronto). The average total nitrogen content of the recombinant Zm cell mass was 13.6% (db).

Determination of growth and fermentation parameters

The mass-based cell yield (obs. $Y_{x/s}$, where s = sugar) was calculated by dividing the maximum dry cell mass concentration (DCM/L) by the mass concentration of sugar used to achieve the maximum culture density.

Unless stated otherwise, the ethanol (product) yield ($Y_{p/s}$) was calculated as the mass of ethanol produced (final concentration) per mass of sugar added to the medium and was not corrected either for the dilution caused by the addition of alkali during the fermentation or for the contribution from fermentable components other than xylose and glucose.

In general, the average volumetric rate of xylose utilization (av Q_S xyl) was determined by dividing the initial sugar concentration by the total time required to achieve complete depletion of sugar from the medium. The maximum volumetric rate of sugar utilization (max Q_S) was estimated from the maximum slope in plots of sugar concentration versus elapsed fermentation time. The corresponding values of volumetric productivity (Q_p and max Q_p) were calculated by multiplying the values of Q_S and max Q_S by $Y_{p/s}$, respectively.

Carbon balancing and determination of %C recovery

Carbon balances were calculated as described previously (Lawford and Rousseau, 1992 and 1993). The amount of carbon (C) in the sugar added (as carbon and energy source) and end-products is presented as milliequivalents C (MW divided by number of carbons). For cell mass, meqC was determined as the dry wt. cells divided by 0.0246 (Lawford and Rousseau, 1993). Carbon dioxide was not determined directly but was estimated by assuming that 1 mole CO_2 was produced per mole of ethanol. For the purpose of carbon balancing, the elemental composition of the cell mass was considered to be constant with a molecular weight of 24.6 g/mol. The carbon content was assumed to 48.7% and the N content was 13.6%. Apart from cell mass, the major end-products were ethanol and CO_2.

Table 2

Composition of Corn Steep Liquor

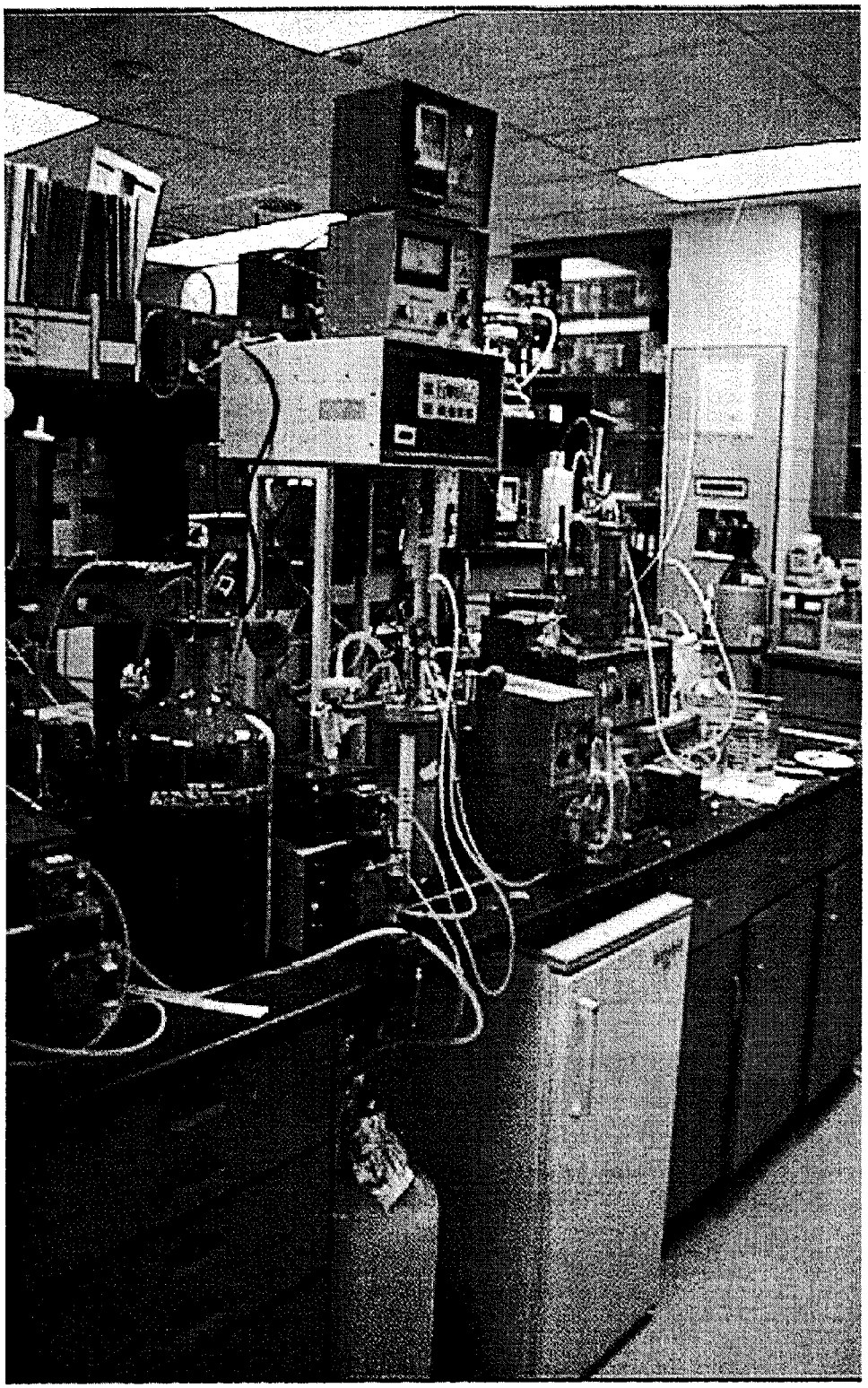
Composition Batch date	cCSL(1/5) 8/97	wCSL 8/97	cCSL(1/5) 7/98	wCSL 7/98
Density	1.035	1.15	1.025	1.12
Percent Solids (w/w)	9.9	47.5	9.5	42.5
Insolubles (% db)	ND	22.1	ND	ND
Protein (% db) Total Kjeldahl N x 6.25	41.8	40.8	43.2	43.1
Lactic acid (% db)	9.5	10.5	11.8	14.4
Vol. Total N (gN/mL)	0.0069	0.0357	0.0068	0.0328

Corn Steep Liquor (CSL) sample supplied by NREL(Golden,Colorado)

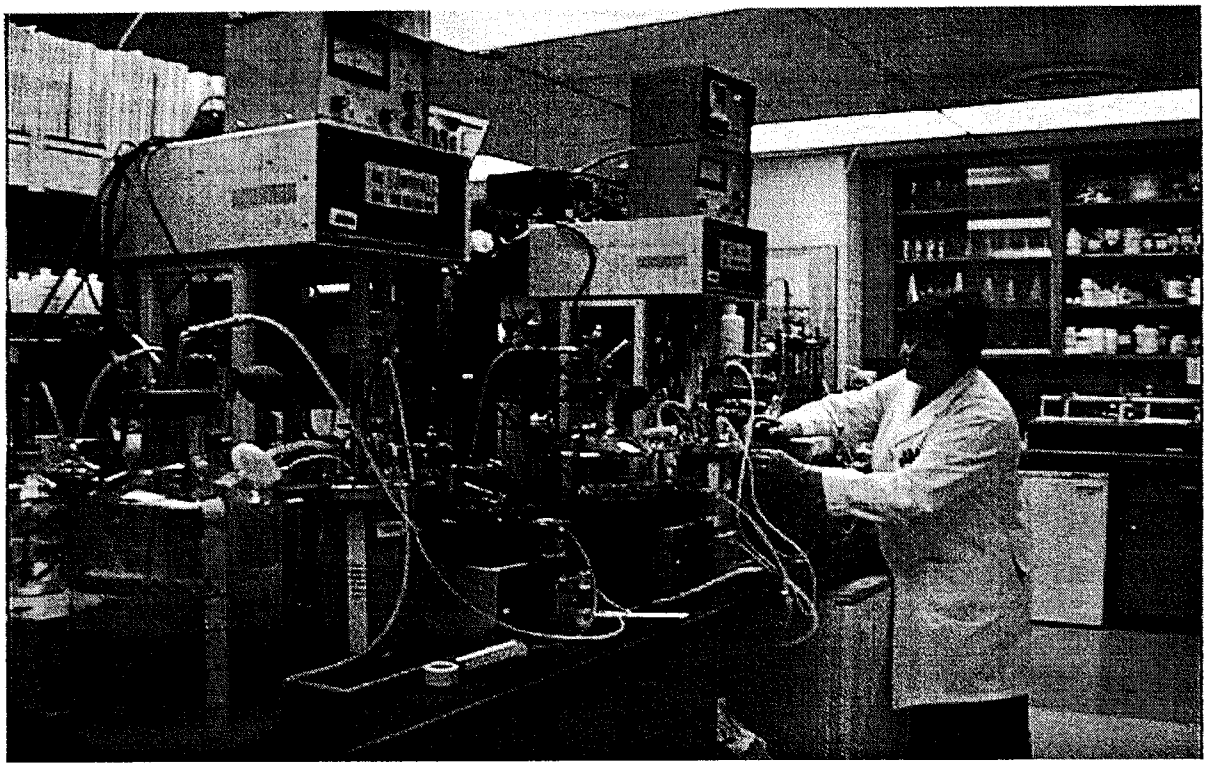
wCSL = whole CSL; cCSL = centrifugally clarified CSL

% db = percent dry basis; ND = not determined

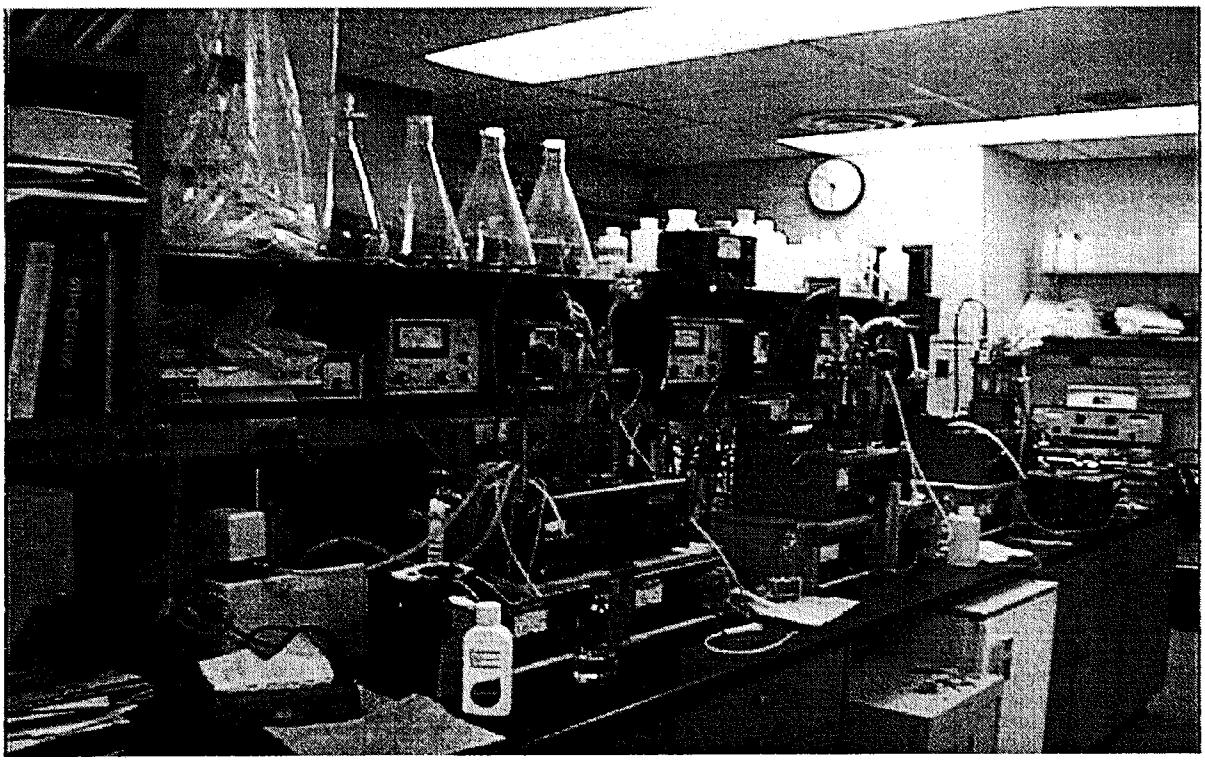
Set-up of NEW Fermentation Equipment (NBS Bioflo 2000)



New NBS Bioflo 2000 fermentors operating in continuous flow mode



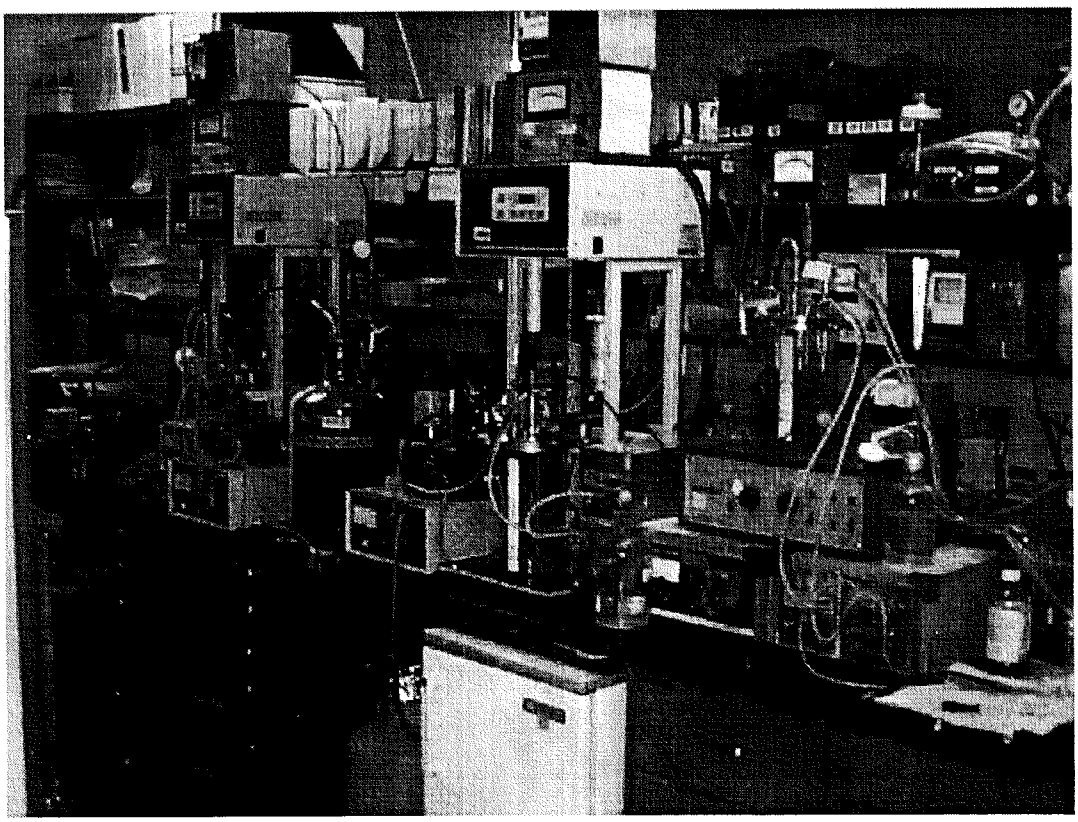
NBS MultiGen F2000 Fermentors operating in the batch mode



Bio-engineering laboratory - NBS bench top bioreactors

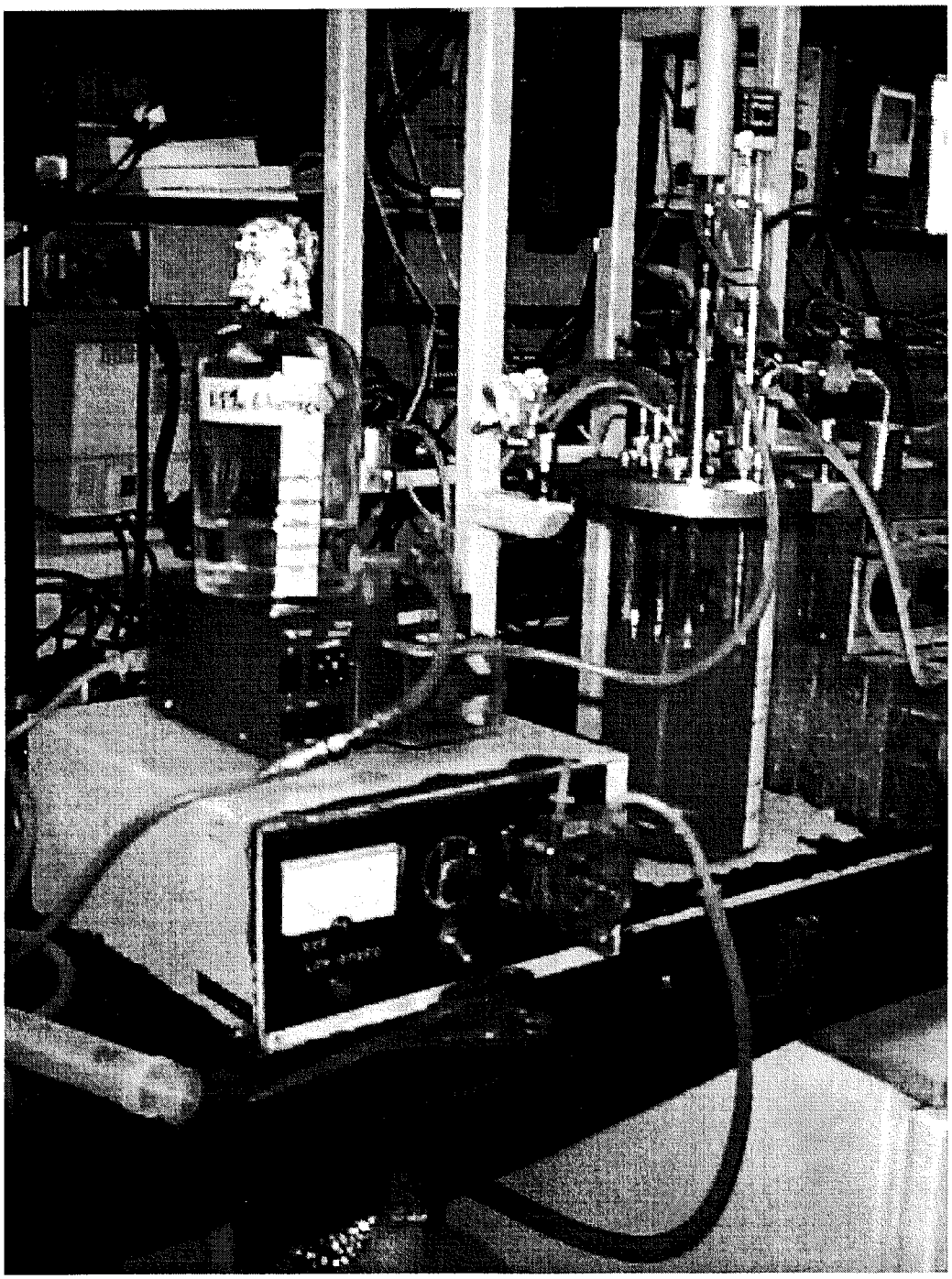
Left: two new NBS Bioflo 2000 units used for continuous fermentations

Right: NBS MultiGen model F2000 used for batch fermentations



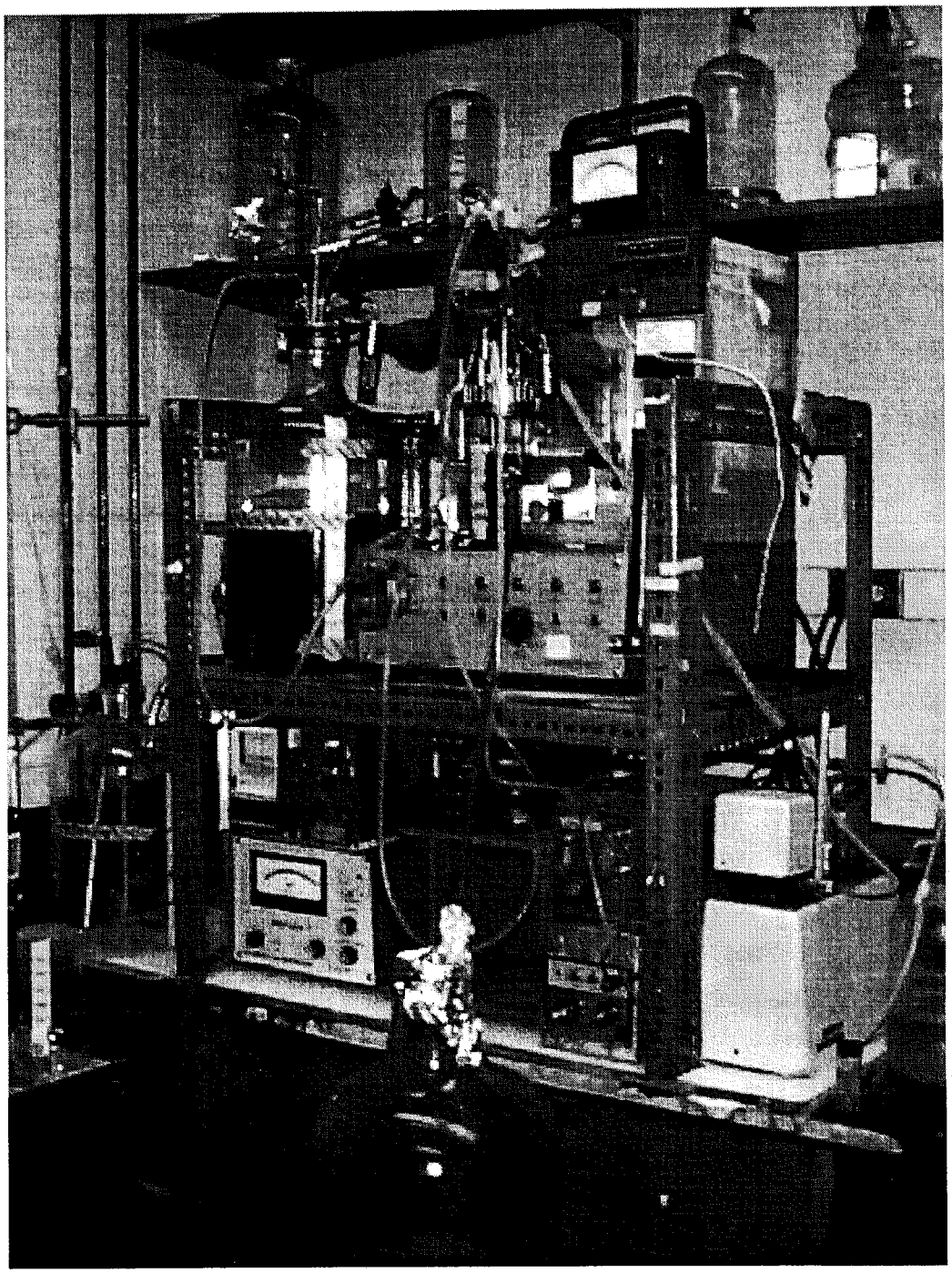
NBS Bioflo 2000 operating as a chemostat

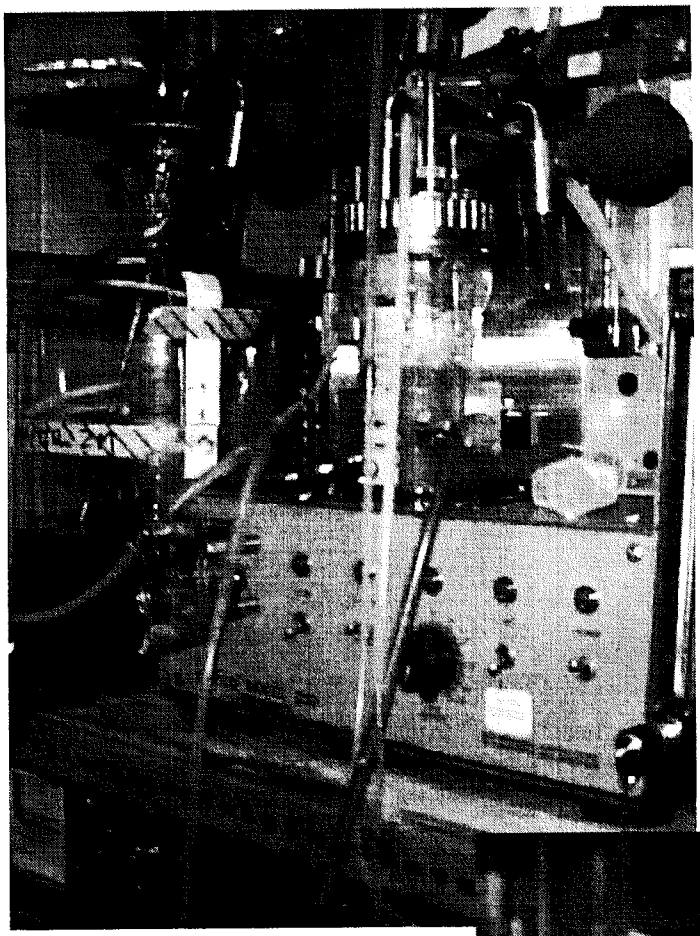
Note: effluent is pumped out and this design may contribute to less wall growth.
Slow feed rates are achieved with the (red) Watson Marlow pump (shown to left)
The working volume is 1500mL.



NBS C30 Bioflo - set up for continuous fermentations

Vessel has sidearm overflow and working volume of 350 mL

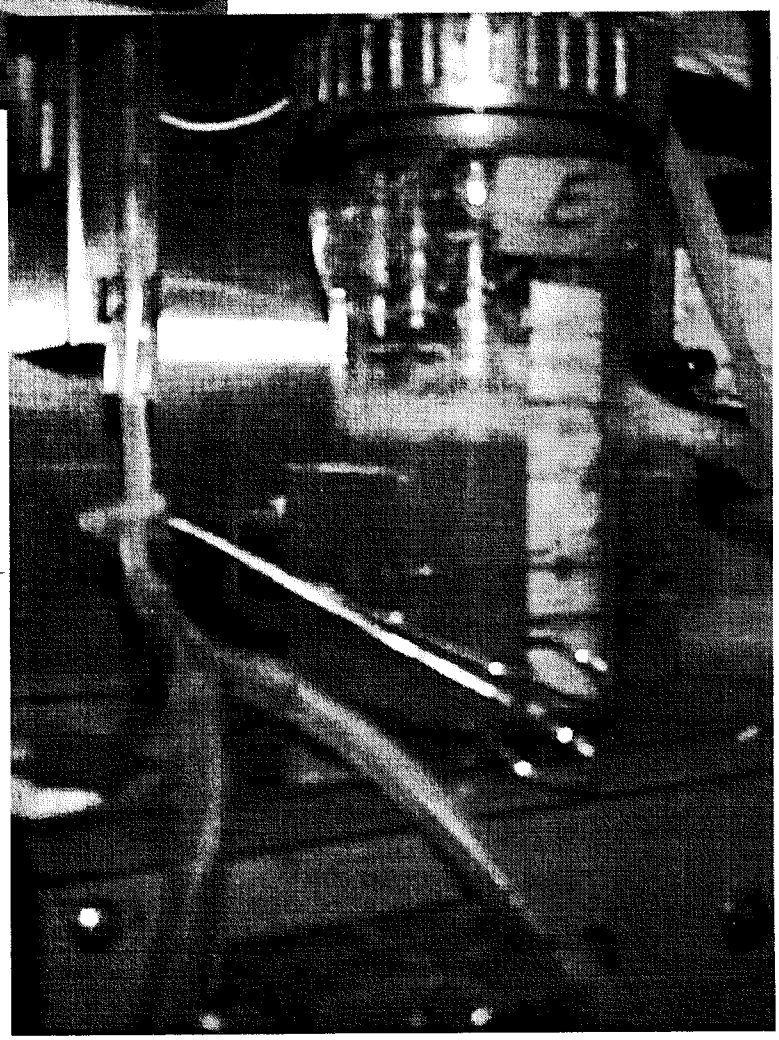




Left **NBS C30 Chemostat**

Constant volume is maintained by sidearm overflow which tends to become occluded by cell mass - this design also has a tendency to prmote wall growth unless the agitation is high

Below **A close up of the vessel** showing wall growth (particularly apparent as a ring of cell mass at the liquid-gas interface) after several days of continuous operation at dilution rate of 0.04/h



RESULTS

and

DISCUSSION

This section is presented in Parts 1 - 6

(see Table of Contents)

PART 1

This part of the work relates toTask #2 of the Statement of Work. The objective was to verify batch fermentation performance of NREL's "adapted" rZ 39676pZB4L strain under standard conditions and to confirm the sufficiency of 2%(v/v) cCSL to support effective fermentation performance.

This work has already been described in the following contexts:

(i) Technical Progress Report #2 (Figs 1,- 4)

(ii) 20th Symposium Paper #76 - see Appendix L (Figs 2 - 5)

Generating the "adapted" variant of rec Zm 39676:pZB4L

In Phase II of this subcontract (1997) we described the cofermentation performance characteristics of recombinant *Zymomonas* 39676:pZB4L in pH-controlled STR bioreactors using synthetic hardwood prehydrolysate media containing pure sugars and acetic acid. Because of the presence of toxic byproducts that are produced during dilute acid-catalyzed hemicellulose hydrolysis, even conditioned prehydrolysates tend to be recalcitrant to fermentation.

The selective pressure exerted on an organism within the controlled growth environment of the continuous flow bioreactor (chemostat) is a powerful research tool for effecting strain improvement through a process of acclimation or adaptation that takes place during the long-term exposure to gradually increasing levels of an inhibitory substance(s).

In generating the strain used in this work the selective pressure provided by the continuous growth environment of the chemostat was exploited to achieve strain improvement through the process of "adaptation" that takes place during the long-term exposure of the biocatalyst to incremental increases in the amount of prehydrolysate in the feed medium. At the beginning of this strain improvement experiment (conducted at NREL) the CSL-based medium contained 10% (v/v) dilute acid-catalyzed yellow poplar prehydrolysate (with pure sugar supplementation to achieve glucose and xylose levels of 0.8% w/v and 4% w/v, respectively). These sugar concentrations were selected because they were similar to the concentrations in full strength hydrolysate liquors obtained by dilute acid pretreatment of hardwood (yellow poplar) sawdust. Batch fermentations had shown that 30% hydrolysate significantly inhibited fermentation performance of the recombinant; however, at the 10% level, performance was not inhibited. The culture that was isolated at the termination of this long-term (149 days) experiment was designated as the "adapted" variant of rec Zm 39676:pZB4L. This hardwood prehydrolysate-adapted strain is the focus of the present work and is referred to as the "adapted" strain.

Comparison of batch cofermentation performance "adapted" variant and non-adapted recombinant 39676:pZB4L in pH-stat STR bioreactors

Batch fermentations with glucose as sole fermentable sugar

The experiment shown in Fig. 1-1, where 4.8% (w/v) glucose was the sole sugar component of a nutrient-rich medium (designated as "ZM" - see *Materials & Methods*), revealed an idiosyncrasy of the "adapted" strain - namely that growth (both rate and yield - Fig 1-1A) and metabolism of glucose (Fig. 1-1B) is slower than with the non-adapted parent culture 39676:pZB4L. This difference might be attributable to changes in the membrane sugar transporter. It is generally believed that the same transporter is responsible for the uptake of both glucose and xylose in *Zymomonas*.

Batch fermentations with xylose as sole sugar component

These experiments were performed because it seemed plausible that a characteristic of the adapted culture, in addition to acetic acid tolerance might be an improved capacity *per se* to utilize xylose. We had observed that in the standard medium (ie. 4% xylose + 0.8% glucose), glucose is utilized more slowly by the adapted strain. We felt that the presence of glucose in the medium might mask any difference in the rate of xylose utilization by the adapted strain. However, Fig. 1-2A shows that growth of the adapted strain in 4% xylose-ZM medium is slower than the parent strain and that the growth yield of the adapted strain is also lower. The final cell mass concentration of the parent strain was 0.68 gDCM/L compared to 0.56 for the adapted strain (Fig. 1-2A). Cell mass production for the adapted strain was further reduced to 0.40 in the 1% (v/v) cCSL medium. Partial replacement of the cCSL by inorganic N resulted in a further lowering of the cell density to 0.19 gDCM/L (Fig. 1-2A). Since it is likely that the energy yield or Y_{ATP} associated with xylose metabolism is less than for glucose, it is not surprising that the growth yield is reduced in a medium where amino N is replaced by inorganic N since more energy would be required to assimilate the latter N source. The pattern of xylose utilization shown in Fig. 1-2B is not simply a reflection of the differences in growth shown in Fig 1-2A. Of particular note is the faster rate of xylose utilization achieved by the adapted strain in the 1% cCSL medium compared to the nutrient-richer ZM medium (Fig. 1-2B). This suggests that CSL has an "uncoupling" effect such that metabolism proceeds faster than growth in the CSL medium. This phenomenon has been observed previously in this lab both with *E. coli* and *Zymomonas*.

Growth and fermentation with synthetic prehydrolysate media (4%X + 0.8%G)

Figure 1-3 compares the growth and cofermentation performance of rec Zm 39676:pZB4L and adapted variant in batch fermentation using a pure sugar nutrient-rich synthetic prehydrolyzate medium with the pH controlled at 5.75. The mineral salts and yeast extract-based ("ZM") medium contained 4% (w/v) xylose and 0.8% (w/v) glucose, but no acetic acid. Under this condition, the performance exhibited by the two strains was remarkably similar (Fig. 1-3). Although the adapted strain produced a lower final culture turbidity (Fig. 1-3A), the final cell mass concentrations were

similar (Table 3). One possible distinguishing feature of the adapted strain revealed in Fig. 1-3B was the apparent slower rate of glucose utilization (see also Fig 1-1) Under these assay conditions (ie. pure sugars - either singly or together), any difference between the two strains was not expected since the medium did not contain any inhibitory substances (principally acetic acid) to which the adapted strain might have become less sensitive.

Comparative cofermentation performance in acetic acid-containing media

Since the adapted strain was isolated from a chemostat that was operating with a feed containing 50% (v/v) prehydrolysate (0.75% w/v acetic acid), it seemed reasonable to assume that altered sensitivity to acetic acid inhibition might be one way to characterise the adapted strain. In previous work we showed the acetic acid sensitivity of rec Zm 39676:pZB4L and showed that 0.4% acetic acid caused a 50% inhibition of growth and cofermentation. Using a CSL-based synthetic prehydrolysate medium containing 0.4% (w/v) acetic acid, the adapted and non-adapted recombinants were compared in pH-stat batch fermentations where the pH was controlled at 5.0, 5.5 or 6.0 (Fig. 1-4). At all three pH values the adapted strain outperformed the non-adapted recombinant with respect to both growth and xylose fermentation; however, the ethanol yield remained close to theoretical maximum for both strains independent of the pH (Fig. 1-4, Table 3). The highest ethanol productivity (0.61g/L/h) was achieved by the adapted strain at pH 6 (Fig. 1-4, Table 3).

Figure 1-5 compares the growth and fermentation performance at pH 6 using the same medium, but with the acetic acid concentration increased to 1% (w/v). At this relatively high level of acetic acid, the adapted strain achieved a slightly higher cell mass concentration (Fig. 1-5A); however, the rate of xylose utilization was significantly faster with the adapted strain relative to the non-adapted strain (Fig. 1-5B). For both strains the ethanol yield (based on sugar consumed) was 0.48g/g (conversion efficiency of 94% theoretical maximum) (Table 3). In the context of rec Zm strain specificity with respect to acetic acid sensitivity, it is interesting to note that our previous work with rec Zm CP4:pZB5 indicates that it possibly rivals the adapted strain in terms of resistance to acetic inhibition.

Comparative fermentation performance of parent and adapted cultures in acetic acid containing ZM medium at pH 6.0

The nutrient-rich "ZM" medium containing 4% xylose and 0.8% glucose was used to test the effect of acetic acid (0.4% and 0.75% w/v) on the non-adapted and adapted rZm cultures. For media containing acetic acid, the pH was controlled at 6.0 rather than at 5.75. Figure 1-6 shows the results of the fermentation series designated as B120.

Table 3 Summary of growth and fermentation parameters

pH	Acetic acid % (w/v)	Maximum Cell Mass (g DCM/L)	Maximum Ethanol (g/L)	Ethanol Yield (g/g)	Ethanol Productivity (g EtOH/L/h)
Non-adapted recombinant					
5.75*	0	1.38	23.6	0.48	0.79
5.0	0.4	0.73	24.0	0.49	(0.35)
5.5	0.4	0.96	24.9	0.50	0.44
6.0	0.4	1.06	24.2	0.49	0.48
6.0	1.0	0.63	15.8	0.48	(0.22)
"adapted" recombinant					
5.75*	0	1.34	23.6	0.48	0.90
5.0	0.4	0.88	24.8	0.50	0.51
5.5	0.4	1.04	24.2	0.50	0.55
6.0	0.4	1.18	24.3	0.49	0.61
6.0	1.0	0.72	21.2	0.48	(0.29)

* The medium was "ZM" with 4% (w/v) xylose and 0.8% (w/v) glucose. All other fermentations were with 1% (v/v) clarified CSL-based media (see *Materials & Methods*) with the same sugar concentrations.
Brackets around values for Ethanol Productivity indicate that xylose utilization was incomplete when batch fermentation was terminated

The values of the maximum cell mass concentrations are given in Table 3B. Fig. 1-6A shows that, in ZM medium in the absence of acetic acid ("control"), the parent and adapted strains display very similar patterns with respect to xylose utilization, although as was observed for the CSL medium, the rate of glucose utilization is slower for the adapted culture (Fig. 1-6). At both levels of acetic acid tested (ie. 0.4% and 0.75% w/v), the rate of xylose utilization was significantly faster with the adapted strain - finishing more than 10h ahead of the parent culture.

Effect of acetic acid on the performance of the 'adapted' rZm in cCSL media

Clarified CSL media containing 4% xylose and 0.8% glucose were used to test the effect of acetic acid (0.4%, 0.75% and 1.0% w/v) on the 'adapted' strain. Figure 1-7 shows the results of the fermentation series designated as B119. The values of the maximum cell mass concentrations are given in Table 3B. In 1% cCSL medium at pH 6.0 the rate of xylose utilization by the adapted strain is very similar with either 0.4% or 0.75% acetic acid with complete utilization of the 4% xylose at 48h (Fig. 1-7A). At 1.0% w/v acetic acid, it takers about 72h to utilize the 4% xylose in the CSL medium (Fig. 1-7A). However, most notable in this series of fermentations is expt B119d in which the level of cCSL was reduced 4-fold without any detrimental affect on the rate of xylose utilization with 0.4% acetic acid in the diammonium phosphate fortified medium (Fig. 1-7B). Hence, it appears that CSL reduction is feasible in an acetic acid-containing medium.

Comparative fermentation performance of parent and adapted cultures in CSL media

Figure 1-8 (expt B118) is representative of several experiments that were performed (see Table 3B for values for final cell mass concentrations). The trajectories for sugar utilization for the parent and adapted strains are shown in Fig. 1-8A and 1-8B, respectively. In the 1% cCSL medium both cultures show almost identical patterns of growth and rate of xylose consumption; however, the adapted strain displays a slower rate of glucose utilization in the mixed sugar media. It is conceivable that this pattern of sugar utilization is a reflection of differences in the affinity characteristics of the sugar transporter in these two cultures. By far the most notable difference in the behaviour of these two strains was observed when the level of cCSL was reduced 4-fold from 1% (v/v) to 0.25% and the medium was fortified with 1.23g/L diammonium phosphate (DAP) to compensate for the decreased level of assimilable N (Fig. 1-8). This level of DAP by itself is sufficient to support approx. 2 gDCM/L based on 13% N in cell mass (eg. see Table 8 of Final Technical Report for s/c AAP-4-11195-03 Aug 30/95). It appears that the adapted strain utilizes xylose at a faster specific rate in a medium of this formulation. Therefore, using the adapted strain, the concept of CSL reduction seems feasible Since the cost of CSL has been shown to have a significant ecomonic impact, this observation is of particular importance.

Table 3B Maximum cell mass concentration for recombinant Zm 39676:pZB4L in different media

	rec Zm 39676:pZB4L			"**Adapted**" 39676:pZB4L		
Medium Composition (pH)	ZM	1%CSL	0.25%CSL + N	ZM	1%CSL	0.25%CSL + N
pH **5.75**						
4%X + 0.8%G	1.38	1.18	1.05	1.34	1.06	1.01
pH **6.0** { + acetic acid }						
4%X + 0.8%G + 0.40% Ac	0.88			0.87	0.87	0.91
4%X + 0.8%G + 0.75% Ac	0.70			0.79	0.81	
4%X + 0.8%G + 1.00% Ac					0.66	

1. Values represent maximum cell mass concentrations as determined by ultrafiltration
2. Variation between different expt. batch series is believed to be due to the amount and the physiological 'state' of the inoculum
3. "ZM" medium = 5g/L YE + ZM salts ($MgSO_4.7H_2O$, 1.0g/L; $FeSO_4.7H_2O$, 0.01g/L; citric acid, 0.21 g/L) + KH_2PO_4, 3.48 g/L
4. Clarified CSL (cCSL) medium prepared with distilled water + ZM salts (see above)

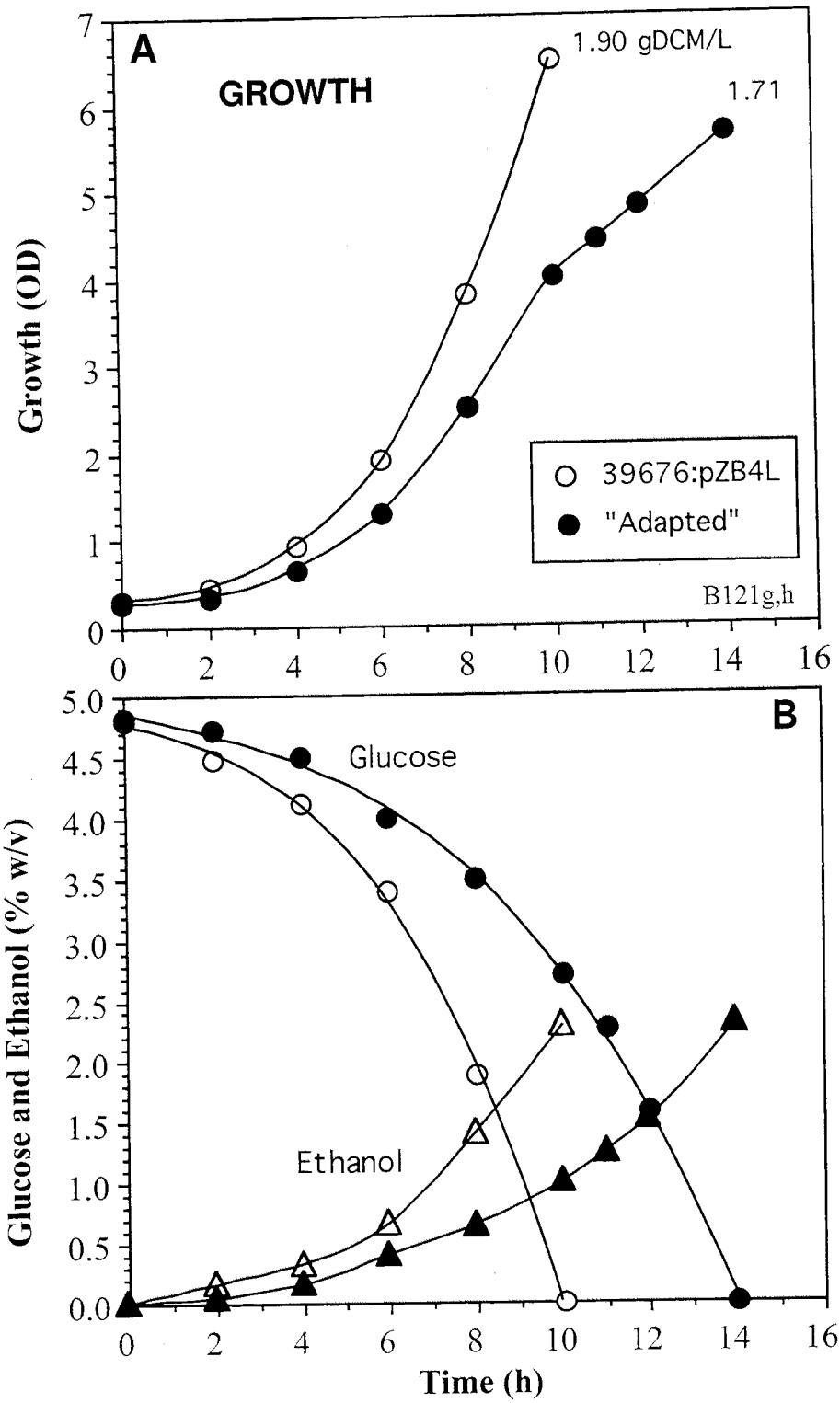


Fig. 1-1 Comparative performance of rec Zm 39676:pZB4L and "adapted" variant with glucose as sole sugar source (**A**) Growth, and (**B**) glucose utilization and ethanol production. The ZM medium contained 4.8% (w/v) glucose (no acetic acid); the pH was 5.75 and the temperature was 30°C. The values for max dry cell mass concentration are shown in panel A

(ref: Fig. 3, 20th Symp. Paper #76 - Appl. Biochem. Biotechnol. 77-79 , 1999)

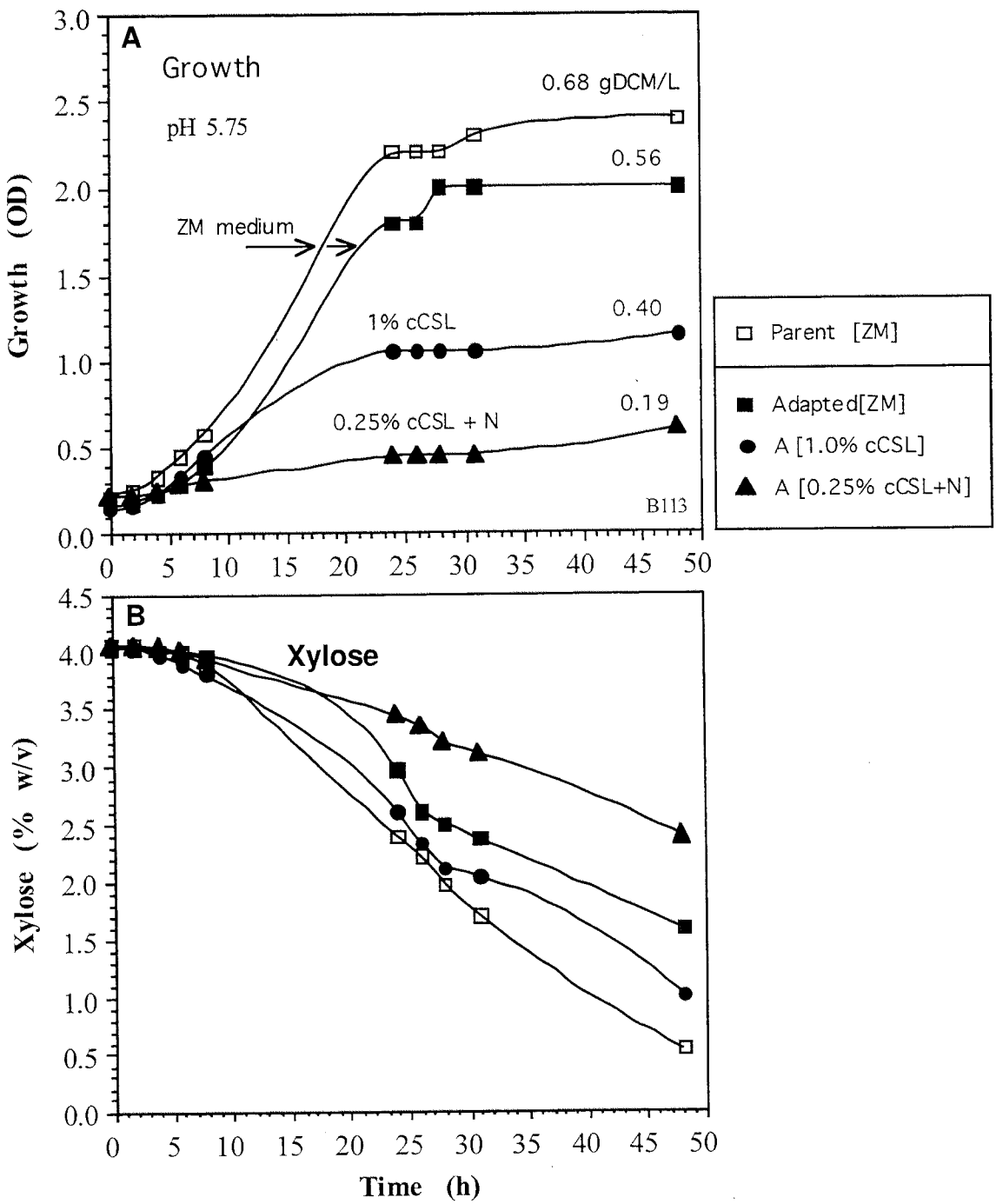


Fig 1-2 Comparative growth and fermentation performance of rZm 39676:pZB4L and the "adapted" variant in ZM and CSL media with **4% xylose** as sole fermentable sugar. Values for max cell mass are given in panel A

(ref: Fig. 1, Prog Report #2 - Feb. 4/98)

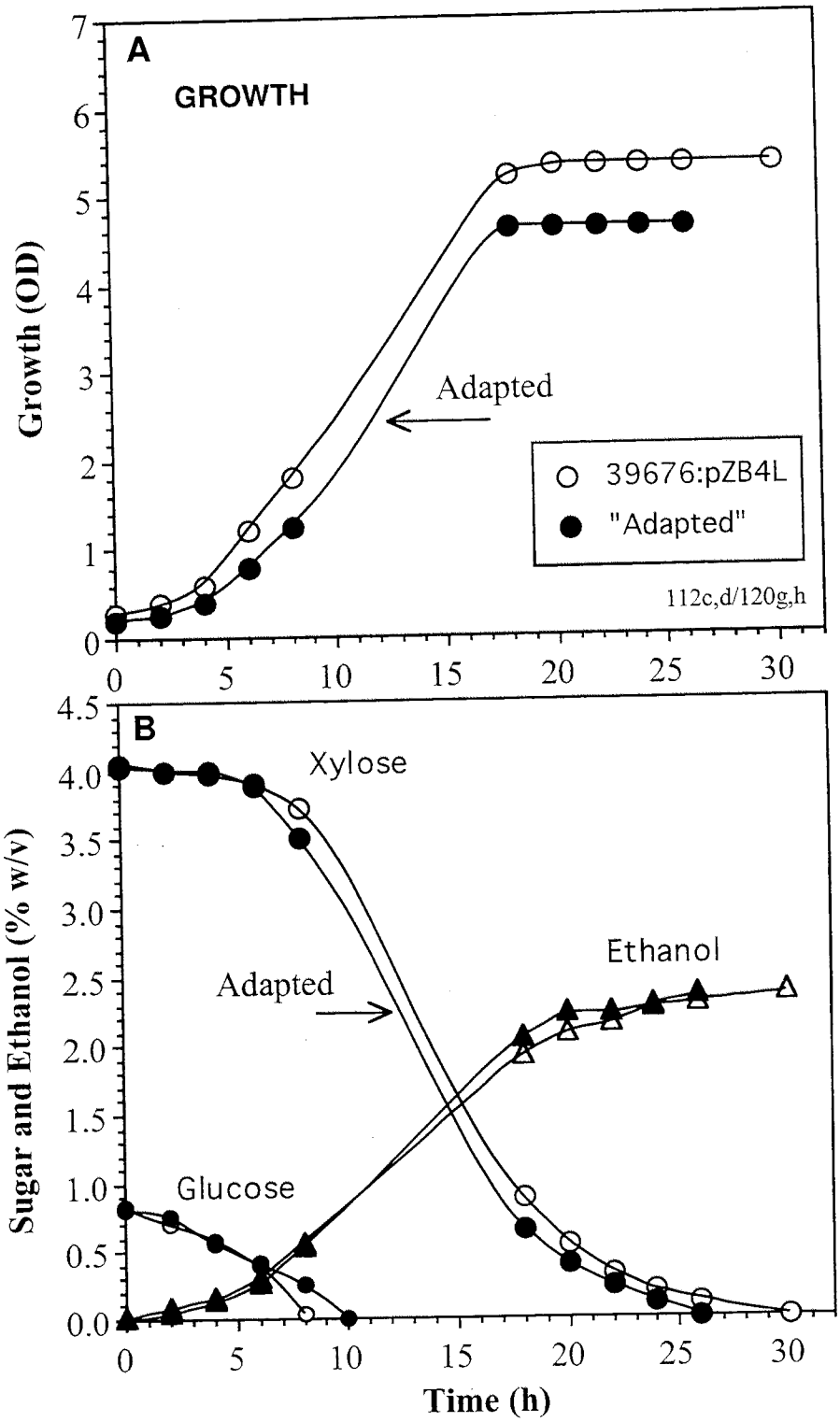


Fig. 1-3 Comparative performance of rec Zm 39676:pZB4L and adapted variant in pH-stat batch fermentation with a pure sugar nutrient-rich synthetic prehydrolysate medium (**A**) Growth, and (**B**) sugar utilization and ethanol production. The ZM medium contained 4% (w/v) xylose and 0.8% (w/v) glucose (no acetic acid); the pH was 5.75 and the temperature was 30°C. The values for max dry cell mass concentration, ethanol yield and productivity are given in Table 3

(ref: Fif. 2, 20th Symp. Paper #76 - Appl. Biochem. Biotechnol., 77-79, 1999)

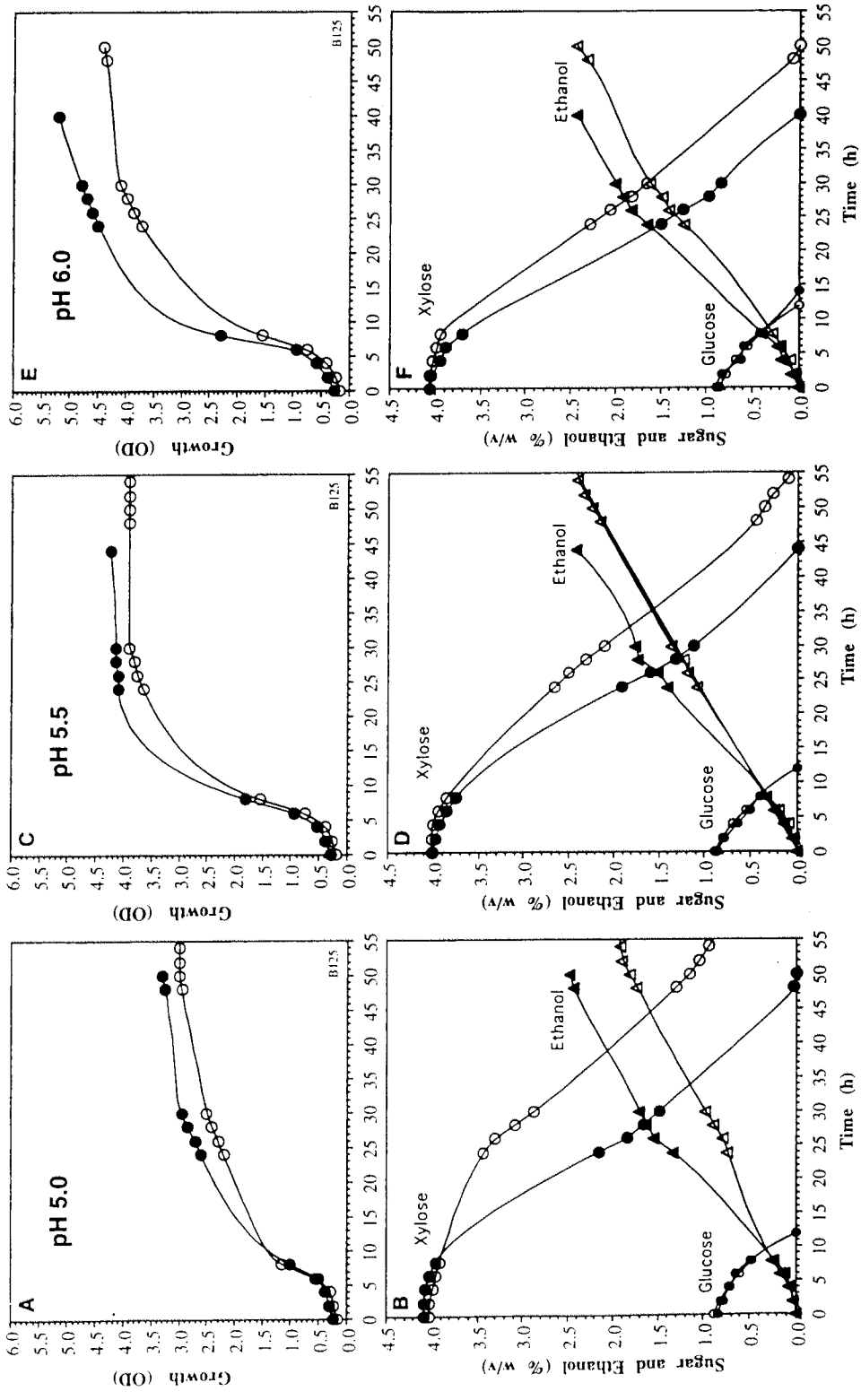


Figure 4

Fig 1-4 Effect of 0.4% (w/v) acetic acid on rec Zm 39676:pZB4L and adapted variant in batch fermentations as a function of pH (**A**) Growth, pH 5, (**B**) sugar utilization and ethanol production, pH 5, (**C**) growth, pH 5.5, (**D**) sugar utilization and ethanol production, pH 5.5, (**E**) growth, pH 6, (**F**) sugar utilization and ethanol production, pH 6. Symbols: O, non-adapted rec Zm; •, adapted recombinant. The mineral salts medium contained 1% (v/v) clarified CSL with 4% (w/v) xylose and 0.8% (w/v) glucose. The values for max dry cell mass concentration, yield and productivity are given in Table 3 (ref: Fig 4 - 20th Symp Paper #76)

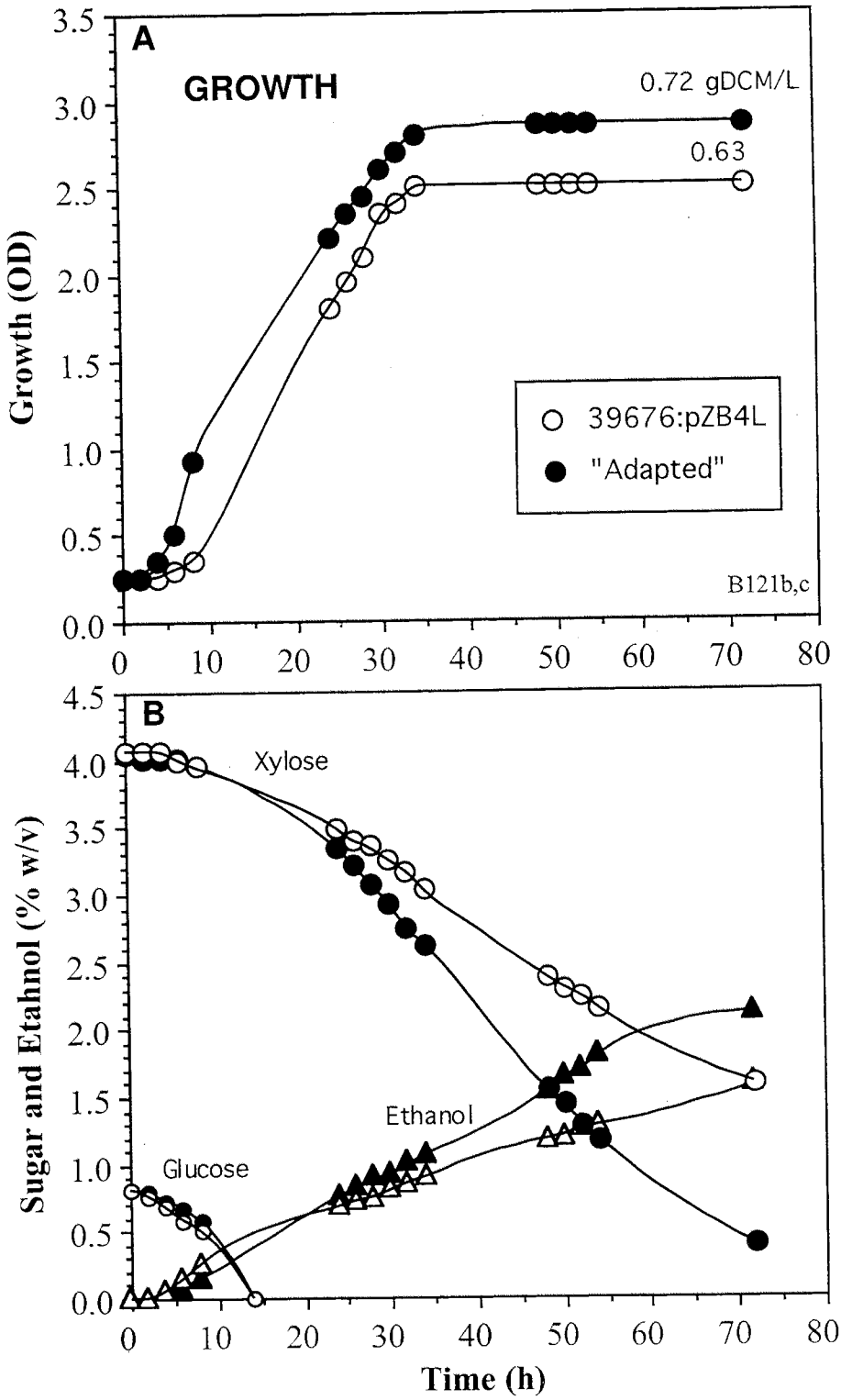


Fig. 1-5 Effect of 1% (w/v) acetic acid on rec Zm 39676:pZB4L and adapted variant in a CSL-based pure sugar synthetic prehydrolyzate medium. (**A**) Growth, (**B**) sugar utilization and ethanol production. The pH was 6 and the temperature was 30°C. The values for max dry cell mass concentration, ethanol yield and productivity are given in Table 3

(ref: Fig. 5, 20th Symp. Paper #76 - Appl. Biochem. Biotechnol. 77-79, 1999)

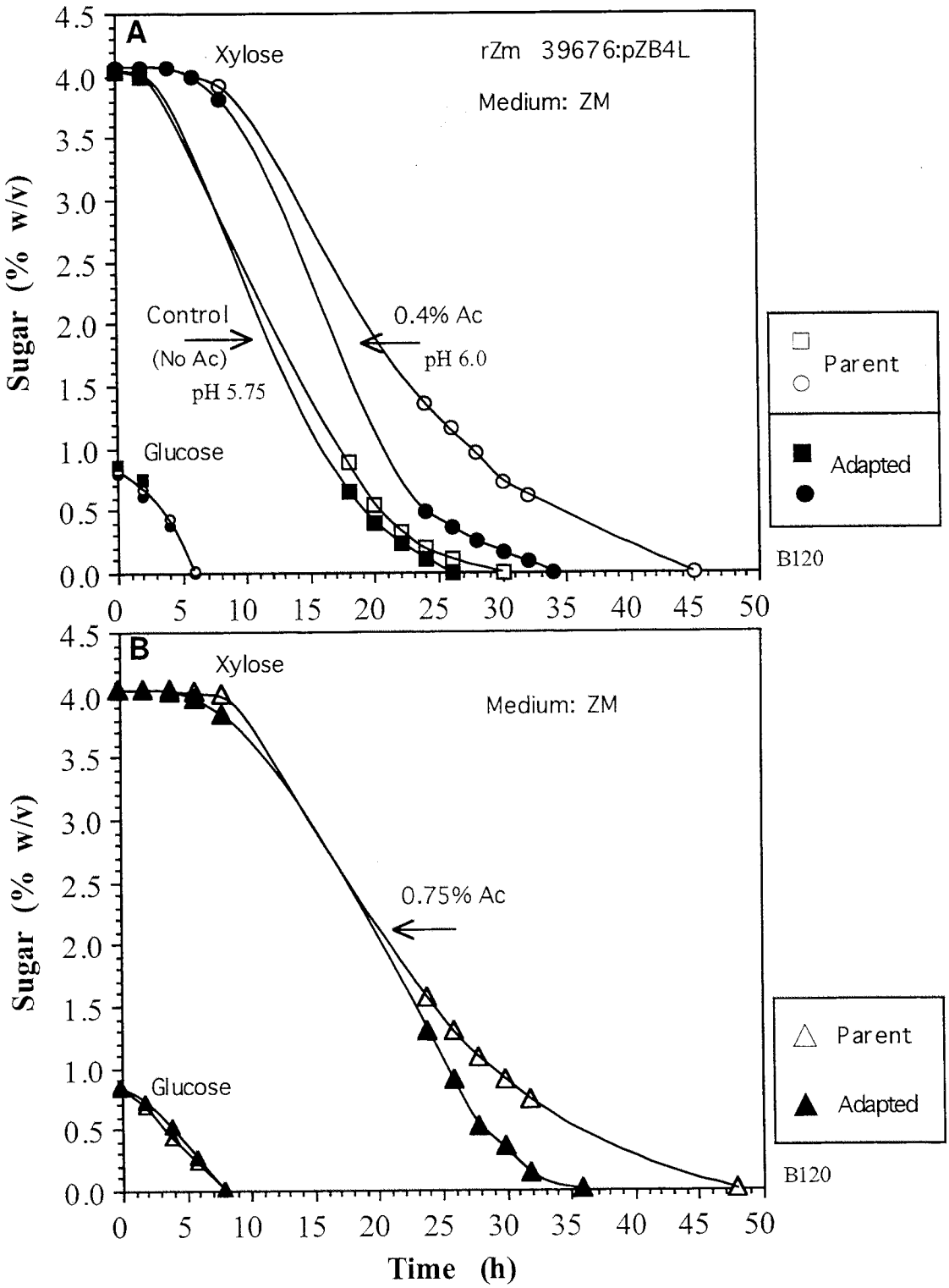


Fig. 1-6 Comparative fermentation performance of rZm 39676:pZB4L and the "adapted" variant in 4% xylose + 0.8% glucose ZM medium with 0, 0.4%, and 0.75% (w/v) acetic acid, at pH 6.0

(ref: Fig. 3, Prog Report #2 - Feb. 4/98)

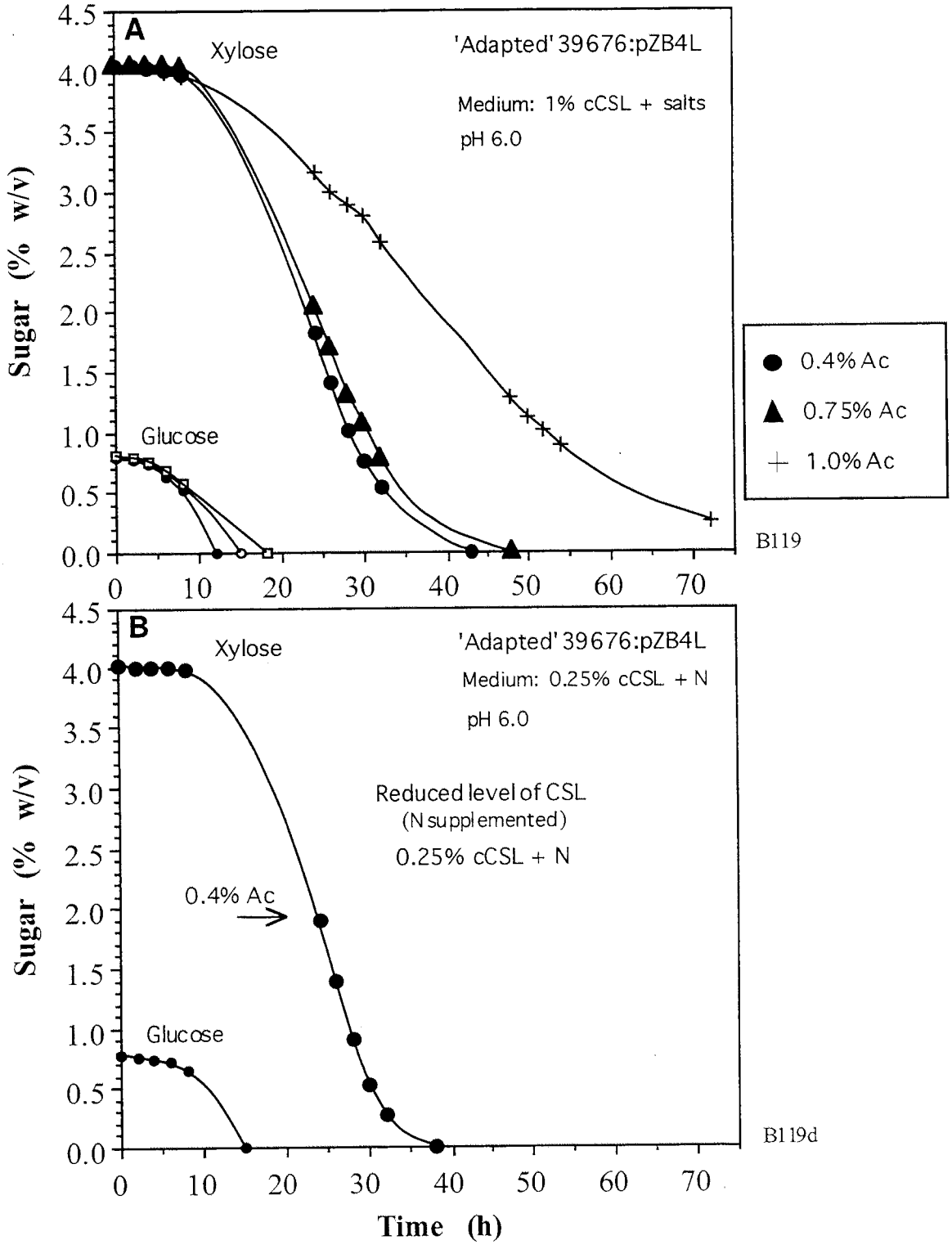


Fig. 1-7 Effect of acetic acid (0.4%, 0.75%, 1.0% (w/v) on the fermentation performance of 'adapted' rZm 39676:pZB4L in cCSL media at pH 6.0

(ref: Fig. 4, Prog Report #2 - Feb. 4/98)

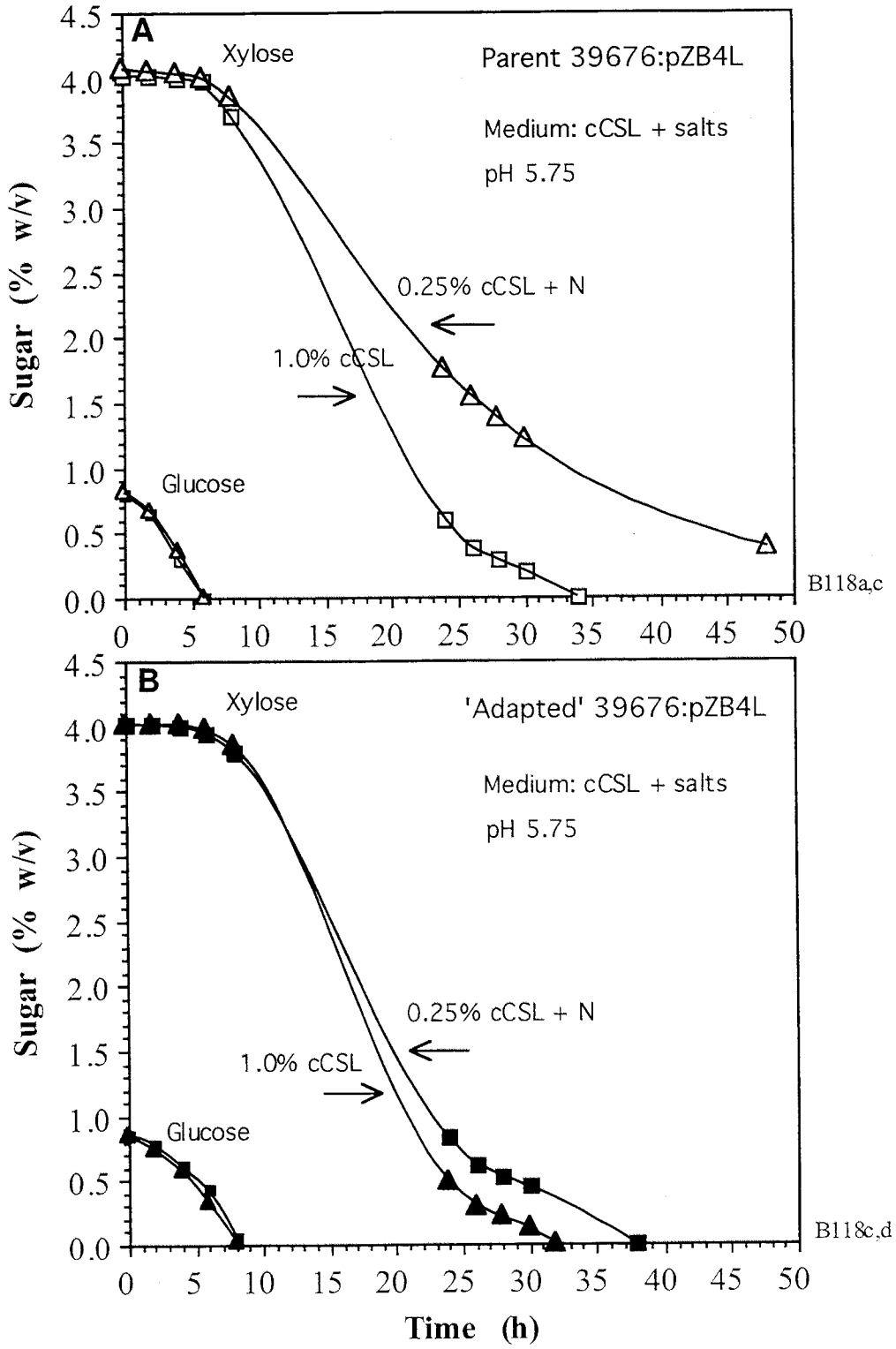


Fig 1-8 Comparative fermentation performance of rZm 39676:pZB4L and the "adapted" variant in cCSL media with 4% xylose and 0.8% glucose

(ref: Fig. 2, Prog Report #2 - Feb. 4/98)

PART 2

The work described in Part 2 is related to Task #3 as outlined in the Subcontract Extension of October, 1998. The objective was to conduct batch fermentations to examine the effect of glucose on the rate of xylose utilization for concentrations of xylose in the range 4%-8%. These experiments will include the use of acetic acid containing media over the pH 5 to pH 6 range.

This work has already been described in the following contexts:

(i) Technical Progress Reports #5 (Figs 1 and 5) and #7 (Fig. 3)
(ii) NREL Seminar (March 2, 1998) - see Fig. 1
(iii) 20th Symposium Paper #75 - see Appendix K (Figs 2, 3 and 5)

Effect of glucose supplementation on fermentation of 4% xylose + 0.4% acetate by rec Zm CP4:pZB5

In previous work presented at the Nineteenth Symposium on Biotechnology (Colorado Springs, 1997), we showed the beneficial effect of glucose supplementation on the rate of xylose utilization by rec Zm 39676:pZB4L in acetic acid-containing synthetic prehydrolysate media. In this part of the work we focused on another recombinant, namely CP4:pZB5 since we had demonstrated the superior acetic acid tolerance of this recombinant. Figure 2-1 shows the effect of various levels of glucose supplementation on the xylose fermentation performance of CP4:pZB5 using a 1% (v/v) cCSL-based medium containing 4% (w/v) xylose and 0.4% acetic acid at pH 6.0. The medium was prepared with distilled water and also was supplemented with 1.67mM magnesium sulfate (see Part 3 for a discussion pertaining to the role of Mg supplementation with CSL-based media). Fig. 2-1 shows that, under these assay conditions, 1.6% glucose appears optimal and that 2% glucose retards both growth (yield) and productivity. The final cell mass concentrations and ethanol productivities for this experiment are given in Table 4.

Fermentation of 6% xylose supplemented with different amounts of glucose

In this series of pH-stat batch fermentations, we explored the effect of increasing levels of glucose supplementation of a medium containing 6% xylose. In the absence of glucose, the recombinant grows relatively slowly (Fig 2-2A) and the final cell mass concentration is 0.74 g DCM/L (Table 4). Slow growth in the absence of glucose is a recognized characteristic of this recombinant, but it was interesting to note that the final cell concentration with 6% xylose was very similar to what was observed previously with either 2.5% (unpublished results) or 4% xylose. This phenomenon of growth limitation cannot be explained in terms of nutrient limitation since the medium used was nutrient rich and clearly capable of supporting higher cell mass concentrations than the limit of 0.74 achieved with xylose as the sole sugar and energy source. Hence the explanation of this observation remains problematic. We have pursured this subject in a separate study on the

energetics of glucose and xylose utilization by rec Zm to be presented at the 21st Symposium this year.

Supplementation of the 6% xylose medium with glucose resulted in faster growth (Fig 2-2A) and a final cell mass concentration that was proportional to the amount of glucose added (Table 4). In the absence of glucose supplementation, about 1.5% xylose remained unconsumed when the experiment was terminated at 72h, whereas all the xylose was completely consumed in 48h when 0.8% glucose was added to the 6% xylose medium (Fig. 2-2C). The rate of xylose utilization is improved by the addition of 2% glucose to the medium; however, levels of glucose supplementation >2% caused the time required for complete 6% xylose utilization to increase from 48h to >60h (Fig. 2-2C). In the case of supplementation at the level of 4% and 6% glucose, the utilization of xylose may be initially retarded because of competitive inhibition of xylose uptake by glucose. Fermentation of an equial amounts of xylose and glucose is discussed later in this section (see Figs. 2-4 to 2-8).

Fermentation of 8% xylose supplemented with different amounts of glucose

In this series of pH-stat batch fermentations, we explored the effect of increasing levels of glucose supplementation of a medium containing 8% xylose. We were interested to see if the same enhancing effect of glucose on xylose utilization that had been observed with 6% xylose could be achieved at higher levels of xylose.

Supplementation of the 8% xylose medium with 0.8%, 2% and 4% glucose resulted in proportionately higher final cell mass concentrations (Fig. 2-3A, Table 4). With the addition of 0.8% glucose, 1.2% xylose remained unconsumed after 72h whereas with 2% glucose added to the medium, the 8% xylose was completely fermented to ethanol (yield = 0.48g/g) in 72h (Fig. 2-3B). Increasing the amount of glucose from 2% to 3% (not shown) or 4% did not shorten the time required for complete xylose utilization (Fig. 2-3B). With 4% glucose, the final ethanol concentration was 5.7% (w/v) (Fig. 2-3C, Table 4) and this level of ethanol may have contributed to a retardation of xylose utilization towards the end of the fermentation (Fig. 2-3B). It was concluded that xylose utilization can be enhanced by means of glucose supplementation with the level of 2% glucose producing the fastest utilization of the 8% xylose.

In another related experiment, that was part of a separate study (data not plotted), we observed that a mixture of 4% xylose and 8% glucose was completely fermented in 60h with a final ethanol concentration of 5.82% (w/v) and volumetric productivity of 0.97g/L/h (Table 4). With this mixture the final cell density was 2.37 g DCM/L; the 8% glucose was completely fermented in 34h and the 4% xylose was completely utilized in 60h (Table 4). Like the experiment with the 8% xylose and 4% glucose (Fig. 2-3C), the relatively high level of ethanol generated from the 8% glucose and 4% xylose medium may have protracted the time required to complete the fermentation by inhibiting xylose utilization in the final stages of the batch fermentation (50-60h) (results not shown).

In the simultaneous cofermentation (SSCF) process design proposed by NREL the saccharification of cellulose would provide a continuous supply of glucose to the ethanologenic biocatalyst. In order to model this situation whereby the continuous supply of glucose might not be expected to cause the same level of competitive inhibition of xylose uptake produced by the higher levels of glucose supplementation previously tested, we performed a fed-batch experiment in which glucose feeding was initiated after the 0.8% glucose had been consumed in batch mode. The feed reservoir contained 4.85% (w/v) glucose and the feed rate was constant at 2ml/h over the period from 8h to 72h (Fig 2-3; open triangle symbol). In the fed-batch experiment, the level of glucose supplementation was equivalent to 1.28% (w/v) (0.8% + 0.48%). Glucose feeding promoted growth beyond the level observed with the standard BPH medium (Fig. 2-3A); the final cell mass concentration was 1.66 g DCM/L (Table 4). However, perhaps most significant was the observation that the xylose utilization trajectories for the 2% glucose supplemented medium and the fed-batch fermentation were very similar (Fig. 2-3B). In a previous study with recombinant Zm we demonstrated the benefit of providing a continuous supply of glucose for xylose fermentation in reducing the effect of inhibition by acetic acid. The results of the present work with the fed-batch fermentation auger well for the performance of recombinant Zm in the simultaneous saccharification cofermentation process for the production of cellulosic ethanol where the xylose concentration is expected to be in the range 4 to 6%.

Fermentation of equal amounts of xylose and glucose

Fermentation of 6% sugar mixture by strains 39676:pZB4L and CP4:pZB5

For purposes of strain comparison, the experiment with CP4:pZB5 and 6% xylose+ 6% glucose shown in Fig. 2-2 is also shown in Figure 2-4which also contains an experiment with the non-adapted strain 39676:pZB4L. Under this condition, CP4:pZB5 appears superior to 39676:pZB4L by virtue of the faster fermentation of xylose. This may derive in part from the better growth exhibited by CP4:pZB5 (Fig. 2-4A). With CP4:pZB5 complete xylose utilization was achieved after 62h (Fig.2-2B). The final ethanol concentration was 5.9% (w/v) (not shown), which represents an ethanol yield of 0.49g/g or a sugar conversion efficiency of 96% of theoretical maximum (Table 5). Our observations with CP4:pZB5 and the 6% sugar mixture are similar to those reported by Peter Rogers *et al.* (1997) as shown in Fig. 2-5; however, whereas our experiments were performed at pH 5.75, it was noted that their experiment was performed with the pH controlled at 5.0 It is interesting to note that Rogers suggested that for this strain the maximum ethanol concentration was probably about 55g/L (p305).

Fermentation of 6.5% sugar mixture by different strains: "adapted 39676:pZB4L, CP4:pZB5 and ZM4:pZB5

At a sugar-to-ethanol conversion efficiency of 94% (equivalent to an ethanol yield of 0.48g/g), the expected yield of ethanol from a mixture of 6.5% xylose and 6.5% glucose is 6.24% (w/v) ethanol. This level of ethanol is higher than had been observed in our previous experiments. It was not known to what extent the pH might affect either the rate of xylose utilization or the final ethanol concentration. Figure 2-6 compares the growth of the "adapted" variant and strain CP4:pZB5 in a 6.5% mixture of xylose and glucose in a nutrient-rich medium at pH 5.0 and pH 5.75 (with and without 0.4% acetic acid). Of particular note is the difference in growth at pH 5. Strain CP4:pZB5 seems much less affected by the change in pH than the non-adapted 39676:pZB4L (Fig. 2-6). Figure 2-7 shows trajectories for sugar utilization for these same experiments with these two recombinants. The values for ethanol yield and productivities are given in Table 5. Clearly, under these assay conditions, strain CP4:pZB5 is superior to the "adaped" variant - this is especially obvious when the medium contained 0.4% acetic acid (Fig 2-7).

With CP4:pZB5 the fermentation appeared to stall with a residual of about 1% xylose at pH 5.0 (Fig. 2-7B), when the ethanol concentration reached 5.5% (w/v) after 32h (Table 5), whereas at pH 5.75, all the xylose was consumed and the ethanol concentration was 6.2% (w/v) after 48h (Table 5). These experiments suggest that, for this rec Zm strain, the upper limit concentration of ethanol, at which xylose utilization is completely inhibited, is pH-dependent. The experiments leading to the previously proposed limit of ethanol concentration of 5.5% by Rogers (1997) were performed at pH 5.0.

Recently Joachimsthal *et al.* (20th Symposium - ABAB, 1999, *in press*) reported that another strain rec Zm 31821:pZB5 (also known as "ZM4:pZB5") can completely ferment a mixture of 6.5% xylose and 6.5% glucose in 48h (at 30°C and pH 5.0) producing "*in excess of 60g/L*"; "*the yield based on sugars available was in excess of 90% of theoretical*" (Abstract #10). *Z. mobilis* ZM4 (ATCC 31821) is the subject of several patents and is claimed to be superior to other wild-type strains with respect to several key process techno-ethanologenic traits. The volumetric ethanol productivity of 1.25g/L/h exhibited by ZM4:pZB5 is similar to that observed in our present study under similar experimental conditions and sugar loading (Table 5). Although it may well be that, because of its pH optimum and ethanol tolerance, *Z. mobilis* ZM4 proves to be a more robust host for pentose metabolic engineering, the present study points to the importance of making performance comparisons under identical conditions since at pH 5.75 rec CP4:pZB5 appears to perform as well as rec ZM4:pZB5 at pH 5 (Fig. 2-8).

Experimental variation

Throughout this work (and in previous work with recombinant Zm) we have noted considerable variation in duplicate experiments. Under certain conditions the variation is large and troublesome. Table 6 is included to illustrate this variation in batch fermentations made under identical conditions Table 6 shows the extremes in observations with respect to two important fermentation parameters - for growth, the maximum cell mass concentration, and for productivity, the time taken for complete sugar utilization for pure sugar nutrient-rich media of various sugar compositions. These "extremes" are represented in Table 6 as "worst case" and "best case" observations for these parameters. This non-reproduciblity is problematic and attempts to ascertain its origin ahve not been successful. We have examined the physiological state of the inoculum (eg. log phase versus staionary phase culture) but the results were equivocal. This variation seriously compromises strain characterization and in comparative studies with different genotypes (genetic constructs) this experimental variation makes it difficult to state with certainty the superior performance characteristics of any particular recombinant relative to the others tested. This is viewed as a major concern in this study and one that needs to be addressed in future work.

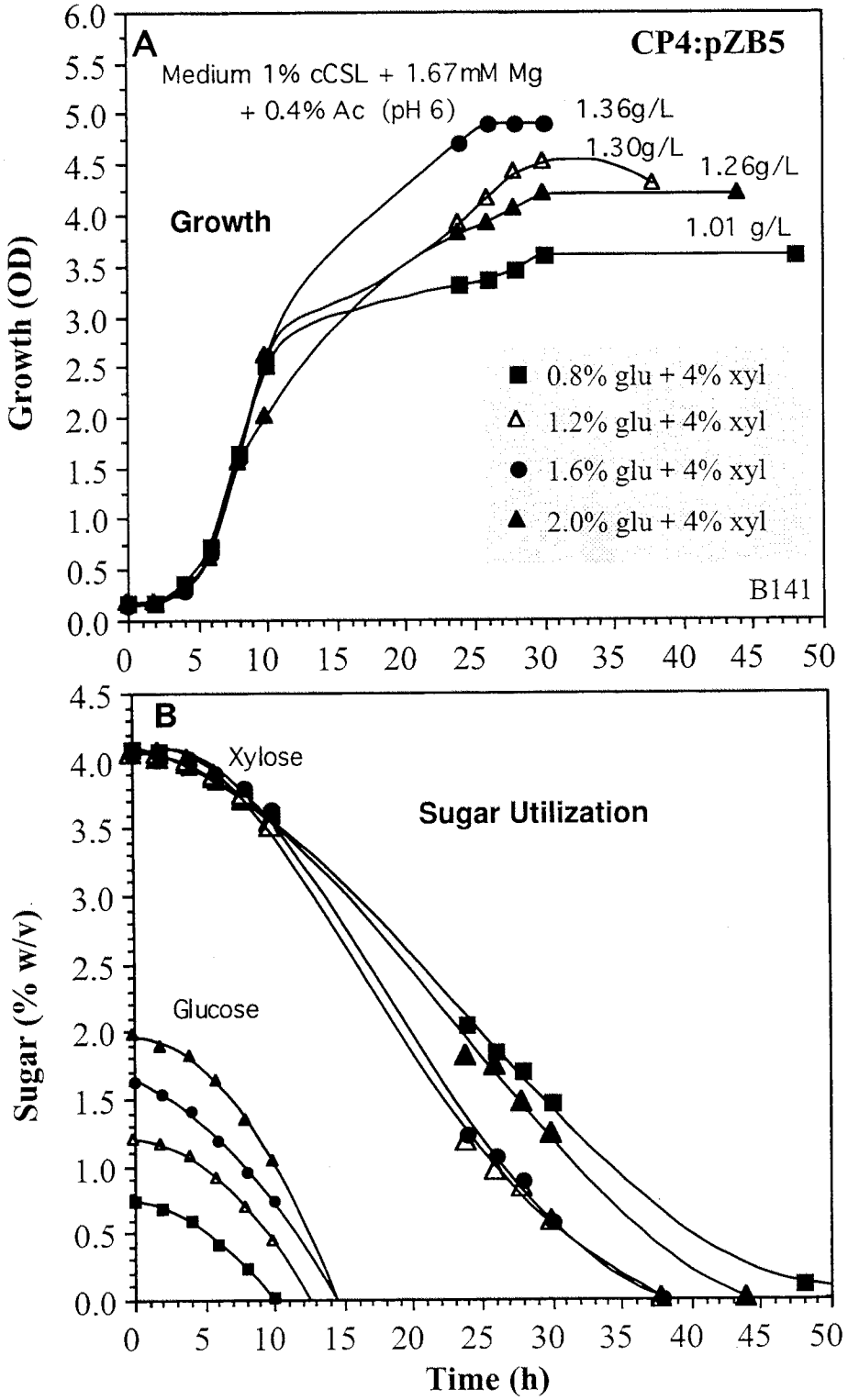


Fig. 2-1 Effect of glucose on fermentation 4% xylose by rec Zm CP4:pZB5 (**A**) Growth, and (**B**) sugar utilization. The 1% cCSL + 1.67mM Mg medium contained 4% (w/v) xylose and glucose in the range 0.8% to 2.0% (w/v) plus 0.4% acetic acid; the pH was 6.0 the temperature was 30°C. Values for ethanol yield and productivity are given in Table 4.

(ref: Fig. 1, Prog Report #5 - Sept. 28/98)

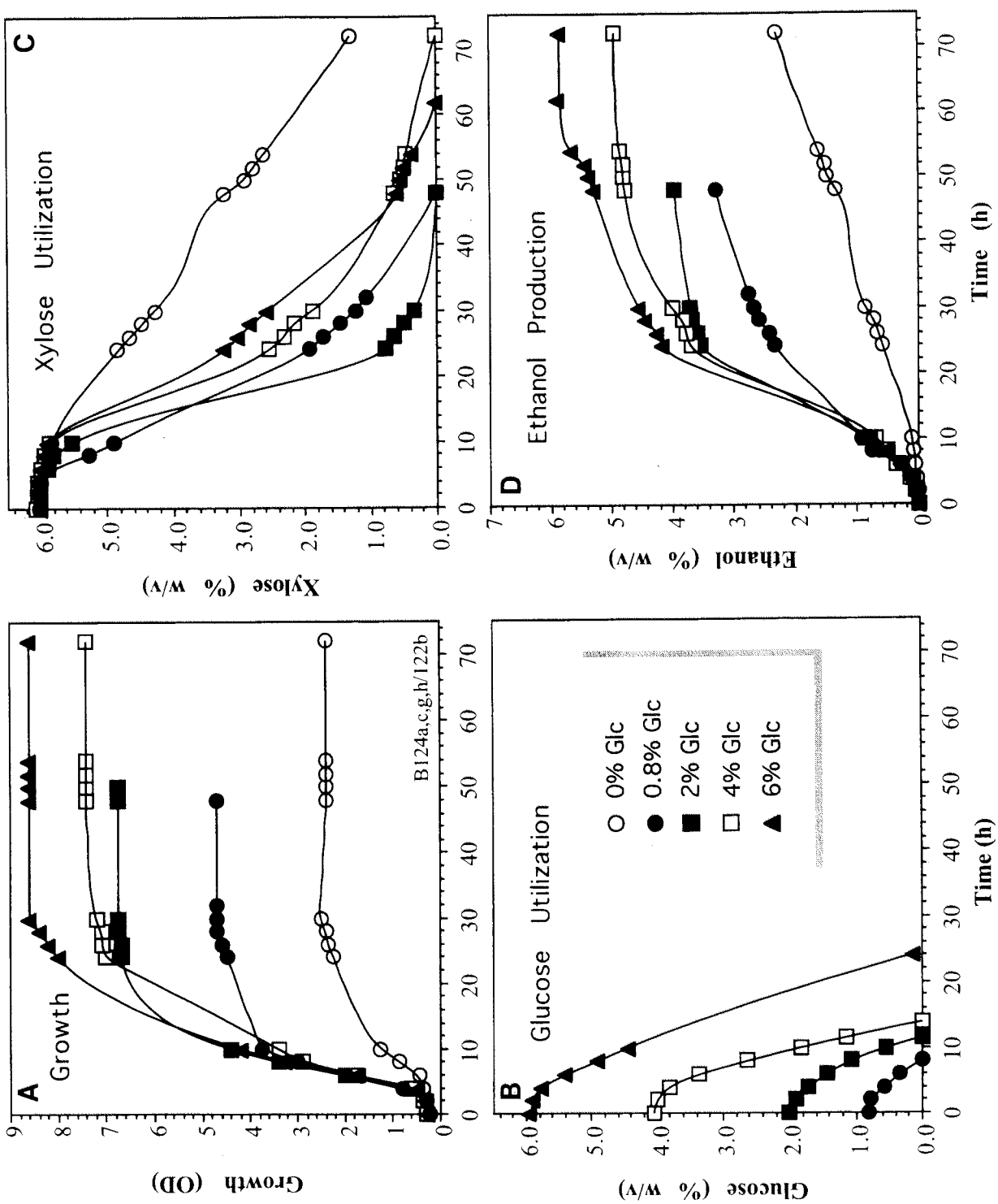


Fig. 2-2 Effect of glucose on fermentation 6% xylose by rec Zm CP4:pZB5 (**A**) Growth, (**B**) Glucose utilization, (**C**) Xylose utilization, and (**D**) Ethanol production. Values for ethanol yield and productivity are given in Table 4.

(ref: Fig. 2 - 20th Symp. Paper #75 - ABAB, ~~70-72~~ 71-79, 1999)

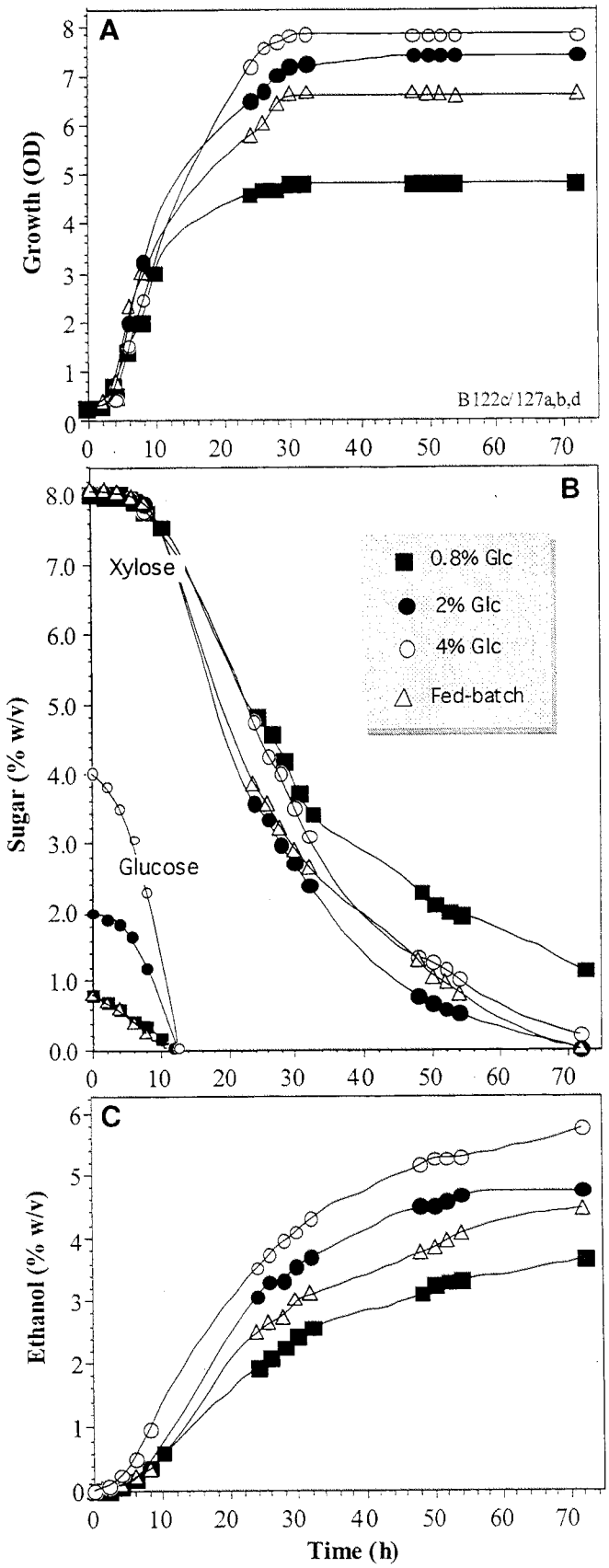


Fig. 2-3 Effect of glucose on fermentation 8% xylose by rec Zm CP4:pZB5 (**A**) Growth, (**B**) Glucose utilization, (**C**) Ethanol production. Values for max. cell mass, ethanol yield, and productivity are given in Table 4. (ref: Fig 3 - Appendix K)

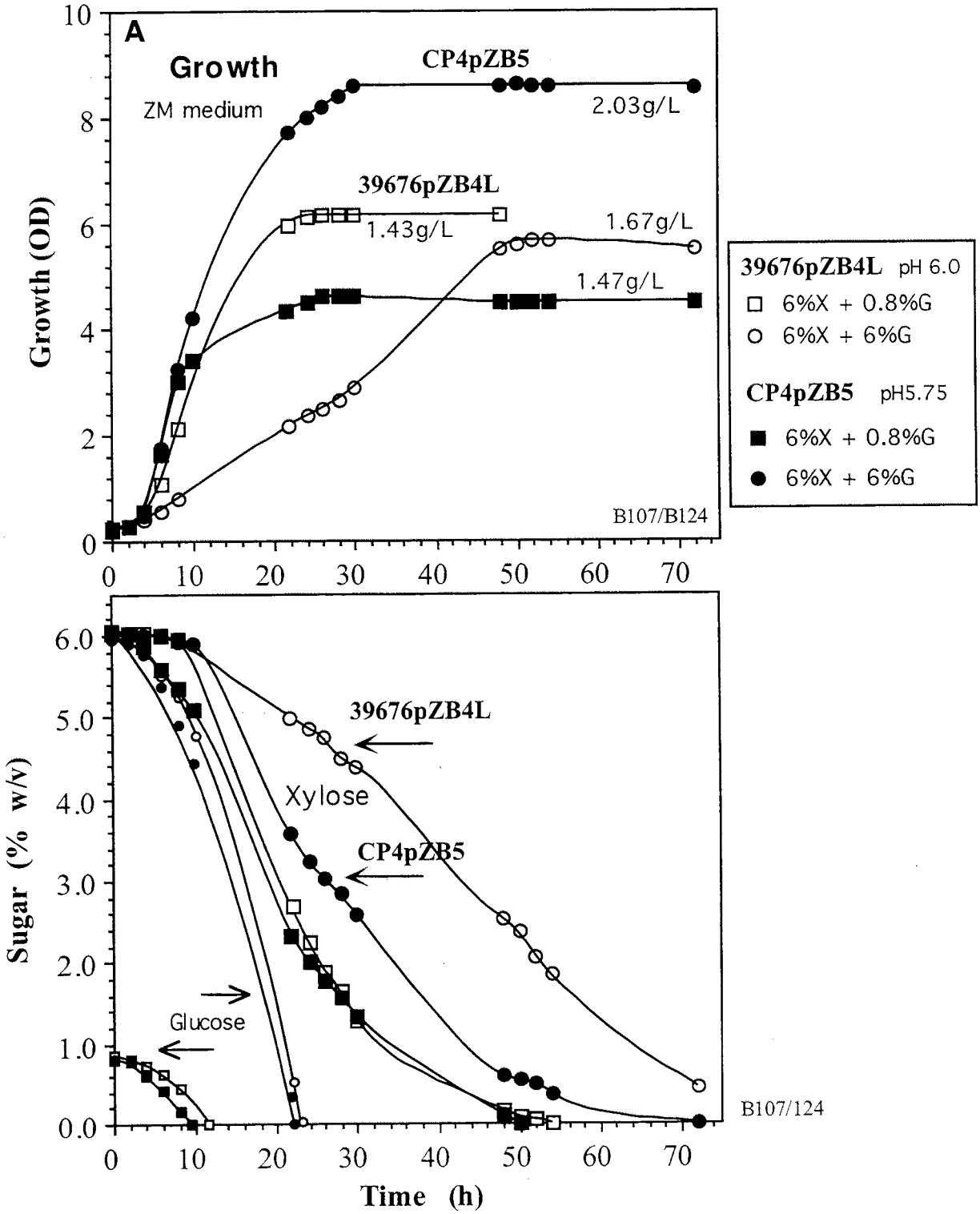


Fig 2- 4 Comparing strains CP4:pZB5 and 39676:pZB4L with 6% xylose and either 0.8% glucose or 6% glucose in nutrient-rich ZM medium. Expts. with CP4 are presented in Fig. 2-2. Values for max. cell mass, ethanol yield, and productivity are given in Table 5.

(ref: "Addendem" to NREL seminar - March 2, 1998)

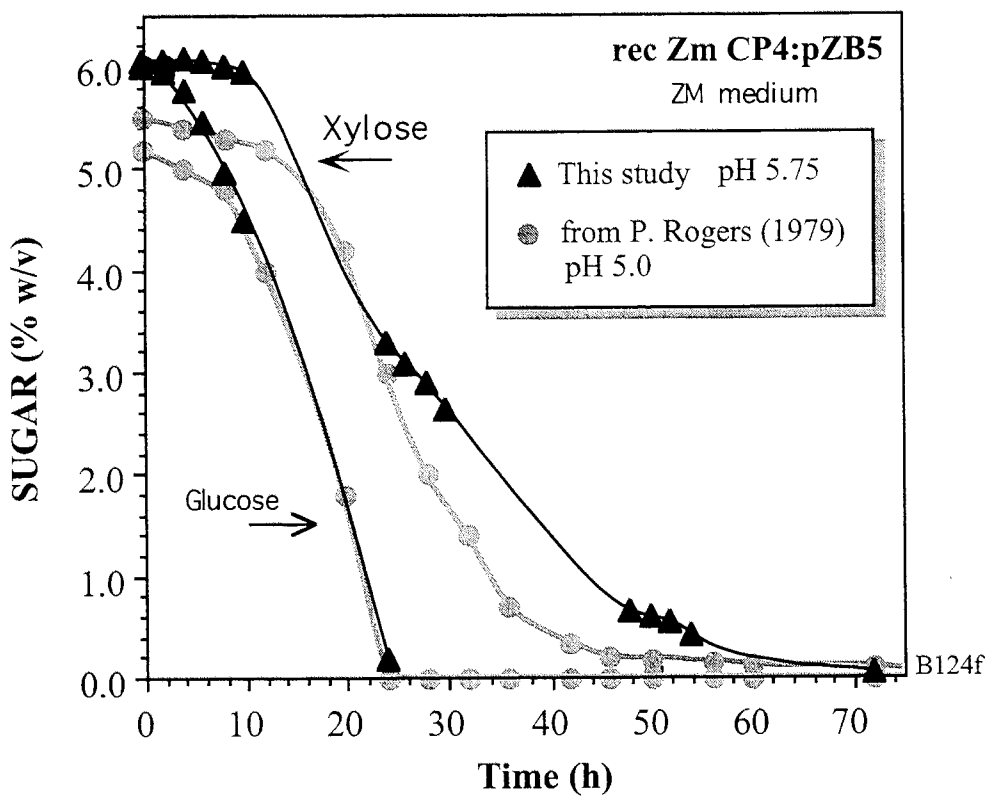


Fig 2- 5 Cofermentation of sugar mixtures (equal amounts of xylose and glucose) by rec Zm CP4:pZB5. Data taken from Figure 1 of P. Rogers *et al.* (1997) *J. Australasian Biotechnol.*, 7, 304-309.
Note: pH 5.0 was used by P. Rogers *et al.*,whereas this work used pH 5.75. Also the amount of sugars is different in the two studies.

(see also NREL Seminar - March 2, 1998)

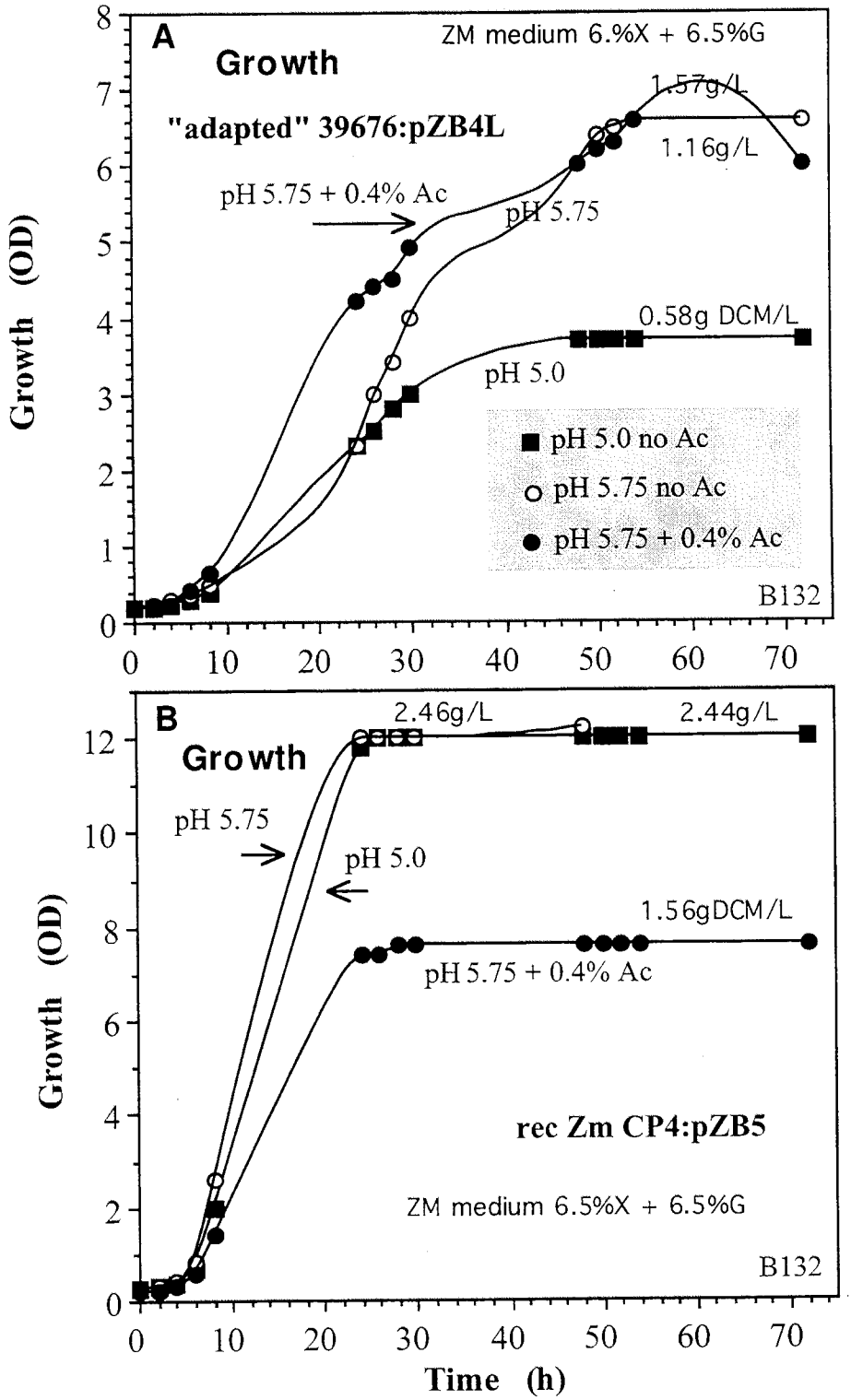


Fig 2- 6 Growth of recombinants CP4:pZB5 and the "adapted" variant in 6.5% sugar mixture at pH 5 and 5.75. (**A**) "adapted" strain, (**B**) CP4:pZB5. Values for max. cell mass, ethanol yield, and productivity are given in Table 5.

(ref: Fig 5, Prog Report #5 - Sept. 28/98)

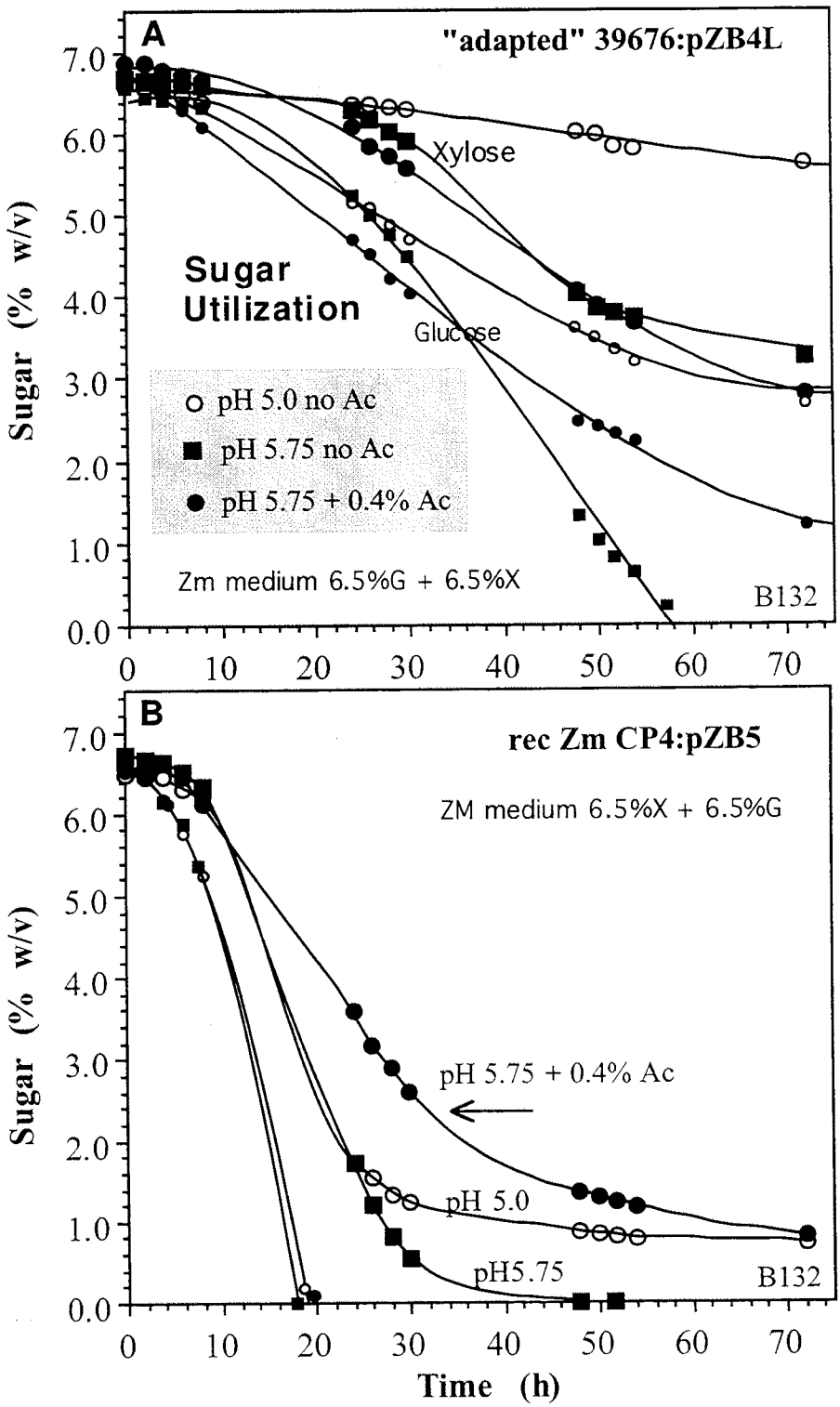


Fig 2-7 Sugar utilization by recombinants CP4:pZB5 and the "adapted" variant in 6.5% sugar mixture at pH 5 and 5.75. (**A**) "adapted" strain, (**B**) CP4:pZB5. Values for max. cell mass, ethanol yield, and productivity are given in Table 5.

(ref: Fig 5, Prog Report #5 - Sept. 28/98; see also Fig. 5 Appendix K)

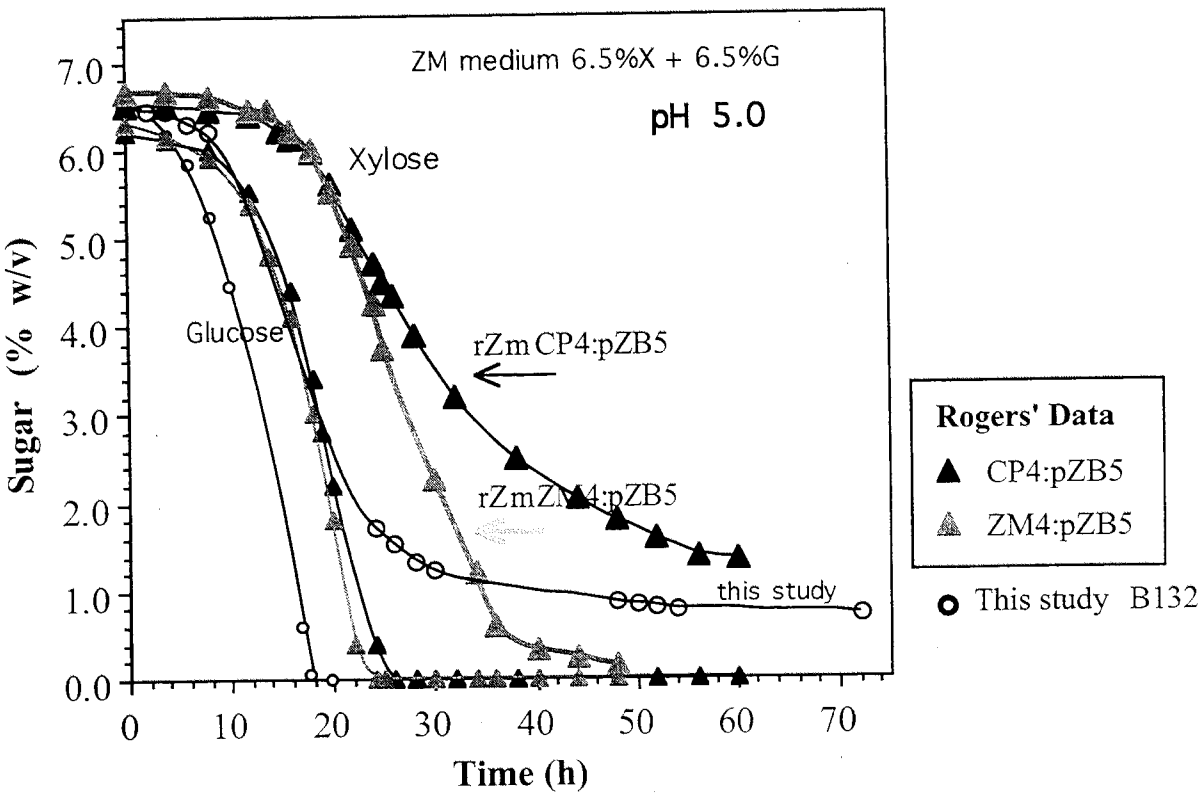


Fig 2- 8 Comparison of this work to published recent data from P. Rogers' lab.

Data taken from Figuress 2 and 5 of Joachimsthal *et al.* (1999) *Appl. Biochem. Biotechnol.* 77-79 (*in press*)
* [presented at 20th *Symposium on Biotechnology*, Gatlinburg, TN, May - 1998]

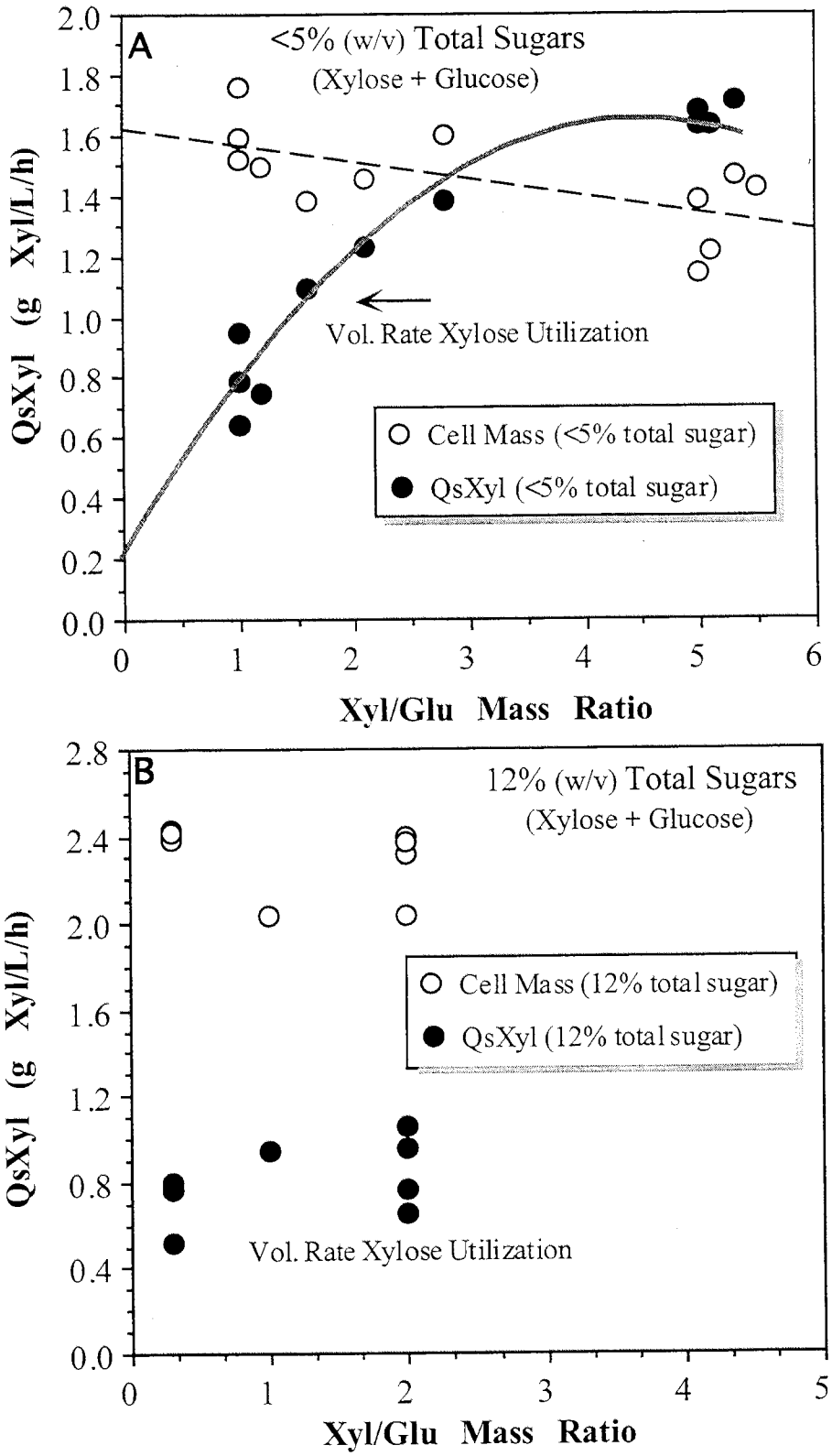


Fig 2- 9 Effect of C5 to C6 sugar mass ratio (specifically Xyl:Glu) on the volumetric rate of xylose utilization [Qs (Xyl) determined as the mass of xylose divided by time for complete fermentation]. (**A**) Expts. where the total sugar was about 4.8% (w/v) as per standard synthetic hardwood prehydrolytsate; (**B**) Expts where total sugar was about 12%

Table 4 Summary of growth and fermentation parameters

Medium (sugar conc'n) Xylose % (w/v)	Glucose % (w/v)	Maximum Cell Mass (g DCM/L)	Maximum Ethanol (g/L)	Ethanol Yield* (g/g)	Ethanol Productivity (48h) (g EtOH/L/h)
Expts for Figure 2-1 CP4:pZB5 (+ 0.4% acetic acid pH 6.0)					
4	0.8	1.01	23.2	0.49	0.48
4	1.2	1.30	26.1	0.50	0.69
4	1.6	1.36	28.7	0.50	0.75
4	2.0	1.26	29.6	0.49	0.67
Expts for Figure 2-2 CP4:pZB5					
6	0	0.74	23.0	0.48	(0.32)
6	0.8	1.47	33.0	0.48	0.66
6	2	1.69	39.7	0.49	0.83
6	4	1.85	49.5	0.49	0.69
6	6	2.03	58.6	0.49	0.95
Expts. for Figure 2-3 CP4:pZB5					
8	0.8	1.48	36.6	0.48	(0.51)
8	2	1.79	47.7	0.48	0.66
8	4	2.03	57.2	0.48	(0.79)
4	8	2.37	58.2	0.48	(0.97)
Fed-batch (0.48 % w/v Glc added over 64h)					
8	0.8	1.66	44.7	0.48	(0.62)

* Yield calculated as ethanol produced per sugar utilized
Productivity based on 48h. Brackets around values for ethanol productivity indicate that xylose utilization was incomplete at 48h

Table 5 Summary of growth and fermentation parameters

Medium (sugar conc'n) Xylose % (w/v)	Glucose % (w/v)	Maximum Cell Mass (g DCM/L)	Maximum Ethanol (g/L)	Ethanol Yield* (g/g)	Ethanol Productivity (48h) (g EtOH/L/h)
Expts for Figure 2-4					
39676:pZB4L					
6	0.8	1.43	33.1	0.48	(0.67)
6	6	1.67	55.5	0.48	(0.94)
CP4:pZB5					
6	0.8	1.47	33.0	0.48	0.66
6	6	2.03	58.6	0.48	(1.10)
Expts. for Figure 2-6 and 2-7 (pH 5 and 5.75)					
"adapted" 39676:pZB5					
6.6	6.7	1.57	46.1	0.47	(0.80)
6.5•	6.5•	0.58	21.9	0.46	(0.30)
CP4:pZB5					
6.6	6.7	2.46	61.9	0.46	1.29
6.5•	6.5•	2.44	58.0	0.47	1.17

• pH 5.0

* Yield calculated as ethanol produced per sugar utilized
Productivity based on 48h. Brackets around values for ethanol productivity indicate that xylose utilization was incomplete at 48h

Table 6 Experimental Variation

Strain	Xyl (g/L)	Glu (g/L)	Worst case		Best case	
			Cell mass (g/L)	Time to complete fermentation (h)	Cell mass (g/L)	Time to complete fermentation (h)
adapted	40	8	1.34	26	1.38	24
39676:pZB4L	40	8	1.41	32	1.41	26
CP4:pZB5	40	8	1.42	30	1.46	24
CP4:pZB5	80	20	1.89	59	2.33	31
CP4:pZB5	65	65	1.70	>72	2.46	48

All experiments were conducted in pH-controlled bioreactors at pH 5.75-6.0 in nutrient-rich media (RM or ZM). More extreme variations were observed with CSL-based media.

PART 3

The work described in Part 3 is related to Task #4 as outlined in the Subcontract Extension of October 1998. The objective was to conduct batch fermentations to examine the requirement for medium supplementation (requirement for DAP and/or Mg) with reduced level of CSL.

This work has already been described in the following contexts:

(i) Technical Progress Report #3 (Fig 1)
(ii) Technical Progress Report #4 (Figs. 2 and 3)
(iii) Technical Progress Report #5 (Fig. 2)

Nutrient studies with 'adapted' rZm 39676:pZB4L

In previous work we have demonstrated that the level of cCSL ammendment could be reduced from 1% (v/v) to 0.25% (v/v) without a significant decrease in performance of the adapted variant (with respect to ethanol yield and productivity in the standard reference medium with 0.4% w/v acetic acid) provided that diammonium phosphate (DAP) or another suitable inorganic N source (eg. ammonium salts, urea, etc.) was added to the medium. However, the medium was prepared with distilled water (dist H_2O) and a cocktail of "Zymo salts" or "Z salts" [1] ($MgSO_4.7H_2O$, 1.0g/L; $FeSO_4.7H_2O$, 0.01g/L; citric acid, 0.21 g/L). In our work with the non-adapted parent culture (39676:pZB4L) conducted in 1997, we used tap water and cCSL without adding any 'salts' since an analysis of tap water (TW) had shown that it contained several of the key trace elements. The analysis of the Toronto TW was included in the Final Technical Report (Aug 30, 1995) - the analysis of tap water is shown below:

Analysis of Toronto Tap Water (U of T Slowpoke Reactor)

Potassium	0.29	mM
Sodium	0.54	mM
Chloride	0.71	mM
Magnesium	0.35	mM
Calcium	1.00	mM
Manganese	0.044	µM
Zinc	0.23	mM
Copper	1.36	µM
Sulphur	1.43	mM

Ref: Final Tech Report Aug 30, 1995

The objective of our recent batch fermentations with the adapted strain was to ascertain the role of the "Zymo salts" in the CSL medium formulation since it was not known to what extent salts supplementation was necessary for robust activity (under the conditions previously used to assess performance - see above). It should be pointed out that there was no acetic acid in the media used in these experiments. The results are described in Figure 3-1. (see also Figs 1-2, 1-7 and Fig 1-8; Table 3B).

From these pH-stat batch fermentations the following can be concluded:

(i) at a reduced level of cCSL (0.25% v/v), Mg supplementation is necessary (Fig 3-2A). The level of Mg required is probably much less than the 4mM used and is probably in the range 1-2mM based on the apparent capacity of Toronto tap water (TW) to supply trace elements (see Fig. 3-3).

(ii) tap water (Toronto) supplies sufficient inorganic elements to achieve fermentation performance that is equivalent to the medium containing the Zymo salts supplement (see Fig 3-2B). In fact the highest cell mass achieved was with the TW medium (Fig 3-1A).

(iii) when 1.23 g/L DAP is present, the level of cCSL can be reduced about 2.5 fold (ie. from 0.25% to ca. 0.1% v/v) but the productivity is affected at the lower level (Fig 3-1B). However, this experiment indicates a range within which reduction of CSL supplementation might be effected.

With respect to the nutrient study in general the following items are important:

(i) the effect of divalent cations in ethanol fermentations is known; for example, Dombeck, K. M. and Ingram, L. O. (1986) Appl. Environ. Microbiol., 52: 975; Osman, Y. A. and Ingram, L. O. (1985) J. Bacteriol. 164: 173. It is possible that Ca can substitute for Mg and in this respect the detox (overliming) procedure may contribute more than suffient Ca to the medium. The analysis of TW shows that Mg is only 0.35mM, but Ca is 1mM, giving a combined divalent cation concentration of 1.35mM which may be the 'appropriate' (minimum) level (see Fig 3-2A). If supplementation with $MgSO_4$ is not required, there is a major economic impact with anticipated cost savings of approx. 18¢/1000L medium.

(ii) theoretically the level of DAP used should be able to support a cell mass level of approx. 2g DCM/L and it is anticipated that since CSL also supplies N, that this level of DAP could easily be reduced by 50% which would have a major economic impact. The cost of using only 0.6g/L DAP would be about 11¢ per 1000L medium.

(iii) the cost of CSL quoted recently by York, S. W.& Ingram, L. O. (1996) J. Ind Microbiol. 16: 374-376 was 20¢ per Kg of dry CSL (equivalent to 9.1¢/lb or about $90 per slurry ton). Parekh *et al.* (1998) quote a cost for CSL of $55/ton (J. Ind. Microbiol. 21: 187-191) which is very close the value of $50/ton (2.5¢/lb) that we have used in previous studies. However, NREL is currently using a value of 6¢ per lb (whole slurry) which is equivalent to $120/ton (whole slurry) (Mark Ruth - pers communic. June 5/98).

(iv) assuming a cost for CSL of 6¢/lb and a supplementation rate of whole CSL of 1% (v/v) and a sugar loading of 10% (w/v) and an overall sugar-to-ethanol conversion efficiency of 95% ($Y_{p/s}$ = 0.485g/g), the following cost for CSL can be determined:

$1m^3$ (1000L) of CSL-based fermentation medium with tap water would cost $1.32

The cost of CSL would be 8.14¢/US gal ethanol

The cost of Mg supplementation at a level of 1.8mM (used in this work) would be 1.62¢/gal ethanol
If whole CSL is used at the reduced rate of 0.25% (v/v), then it might be necessary to also supplement with DAP. The cost implications are summarized below:

CSL cost	2.5¢/lb	6¢/lb
at supplementation rate of 1% (w/v) whole CSL		
Fermentation medium $1m^3$ (1000L)	55¢	132¢
CSL (¢/US gal EtOH) (1% w/v)	3.39¢	8.14¢
(at reduced level of 0.25% w/v)	0.85¢	2.04¢
DAP at 1.32g/L (only for reduced CSL)	2.93¢/gal EtOH	
MgSO4 at 1.8mM	1.62¢/gal EtOH	

* note: these levels of DAP and Mg are probably maximal and might be reduced where the cell mass concentration is likely to be less than 2gDCM/L

Total cost for nutrients	**¢/US gal EtOH**	
(1% w/v whole CSL + Mg)	5.01¢	**9.76¢**
(0.25% CSL + DAP + Mg)	5.40¢	**6.59¢**

Using the current NREL cost for CSL of 6¢/lb (whole slurry), the cost of using CSL as a growth an fermentation nutrient source is about 8¢ per gallon of ethanol. When Mg supplementation is included th cost rises to about 10¢/gal. Even at a four-fold reduced level of CSL supplementation (0.25% v/v), th cost is estimated at about 7¢/gal. This study indicates that reduced levels of CSL with DAP/M supplementation may be permissible with hydrolysate media (where acetic acid reduces cell mass level and this brings the cost more in line with the current NREL target for nutrients of about 5¢/gal.

Nutrient studies with acetic acid-containing media

We have extended this nutritional study to include media containing 0.4% acetic acid with a view to testing the nutritional requirement under a more stressful condition for growth and fermentation.

Figure 3-3 shows the results of several batch fermentations conducted at pH 6. The results support the contention that magnesium supplementation is important; however, we have shown that the level of Mg can be effectively reduced to about 1.5mM and this has significant economic impact.

Figure 3-4 shows the same type of experiments as Fig 3-3 except the the pH was lowered from 6 to 5.75. The consequence of this reduction in pH on both growth and xylose fermentation in the acetic acid media is quite dramatic and does not auger well for operation at lower pH such as being proposed by Peter Rogers unless one uses a more pH/Ac tolerant variant strain.

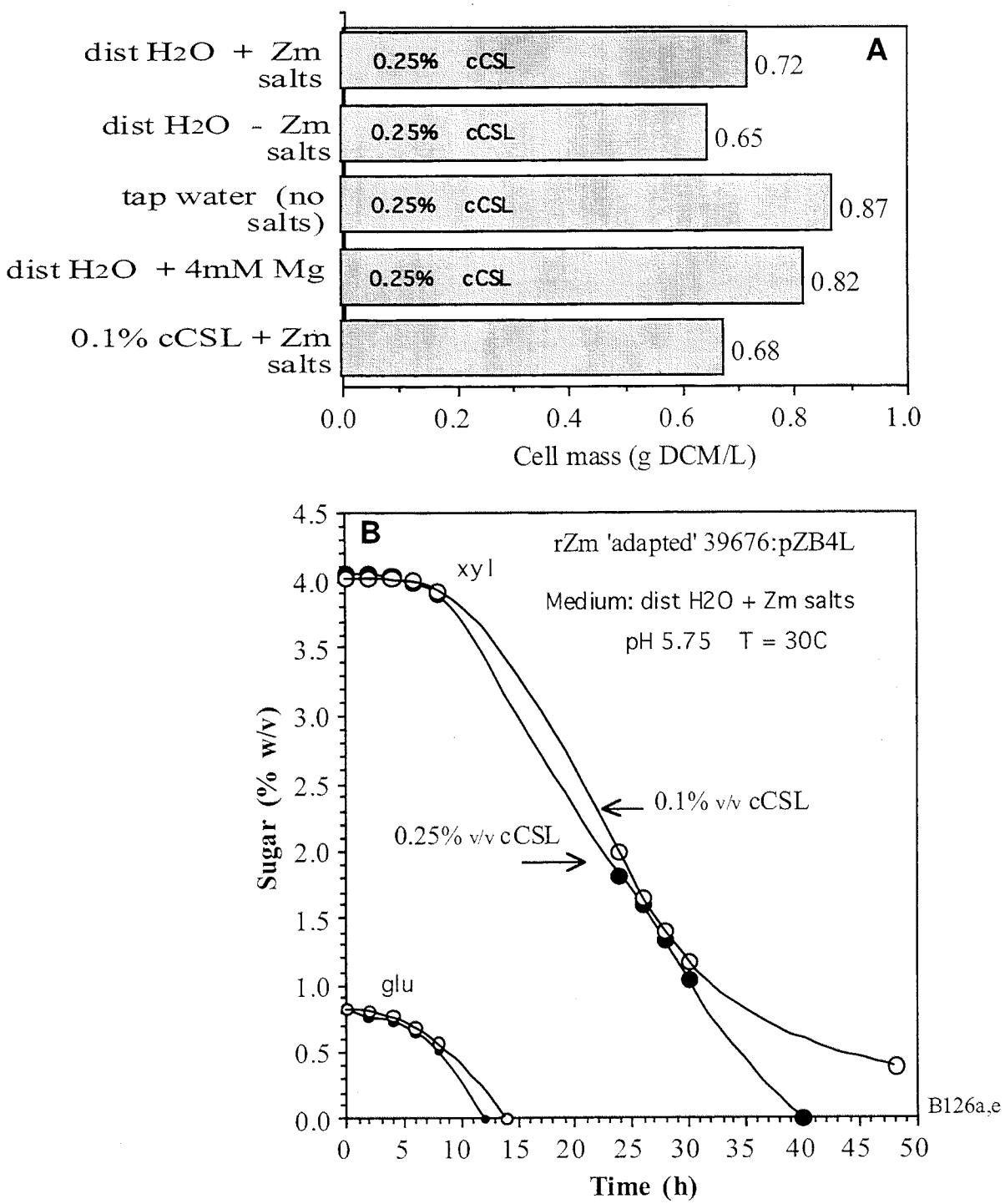
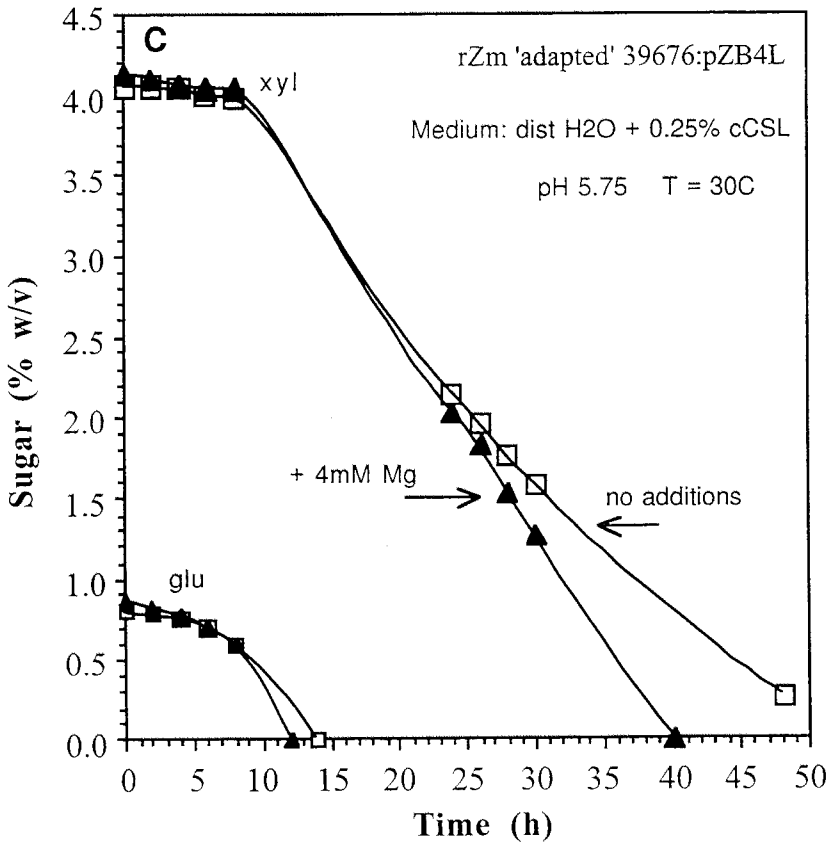


Fig. 3-1 Effect of alterations to CSL-based media with "adapted" strain with 4% xylose + 0.8% glucose at pH 5.75. (**A**) The amount of cCSL in the medium was 0.25% v/v except where it was only 0.1%. " Z salts" = MgSO4.7H2O, 1.0g/L; FeSO4.7H2O, 0.01g/L; citric acid, 0.21 g/L (**B**) Sugar utilization at reduced levels of CSL

(ref: Fig 1, Prog Report #3 - April 1/98)



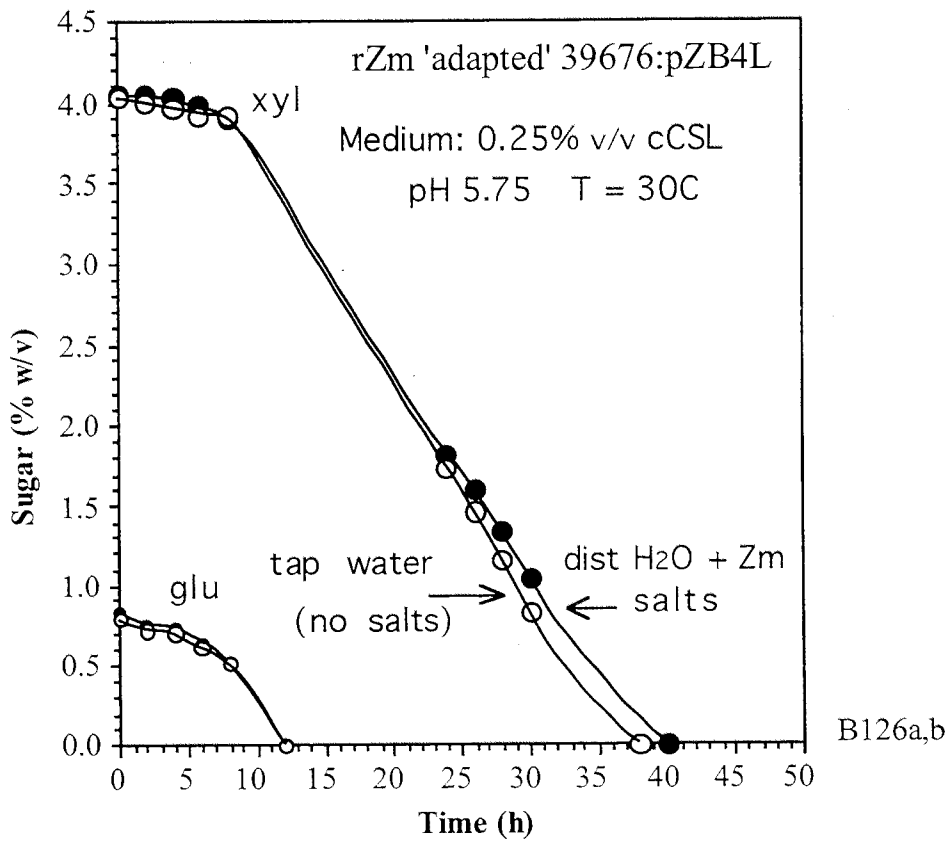


Fig. 3-2 Effect of alterations to CSL_based media with "adapted" strain with 4% xylose + 0.8% glucose at pH 5.75. (see Panel A of Fig 3-1)

(ref: Fig 1C and 1D, Prog Report #3 - April 1/98)

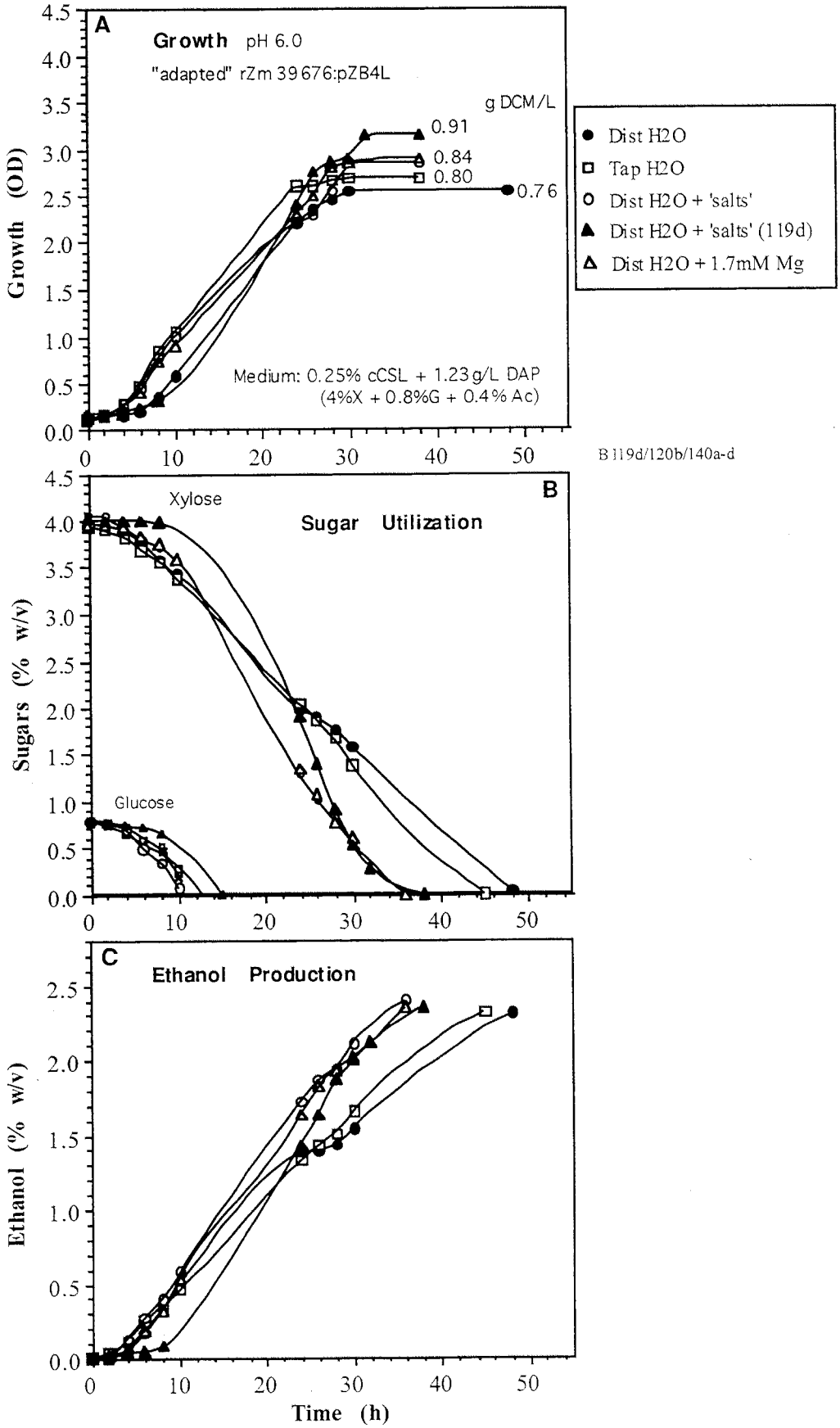


Fig. 3-3 Requirement for salts supplementation with a 0.25% (v/v) cCSL + 0.4% acetic acid medium (**pH 6.0**) and rZm "adapted" 39676:pZB4L [Fig 2, Report #4 - July 23/98]

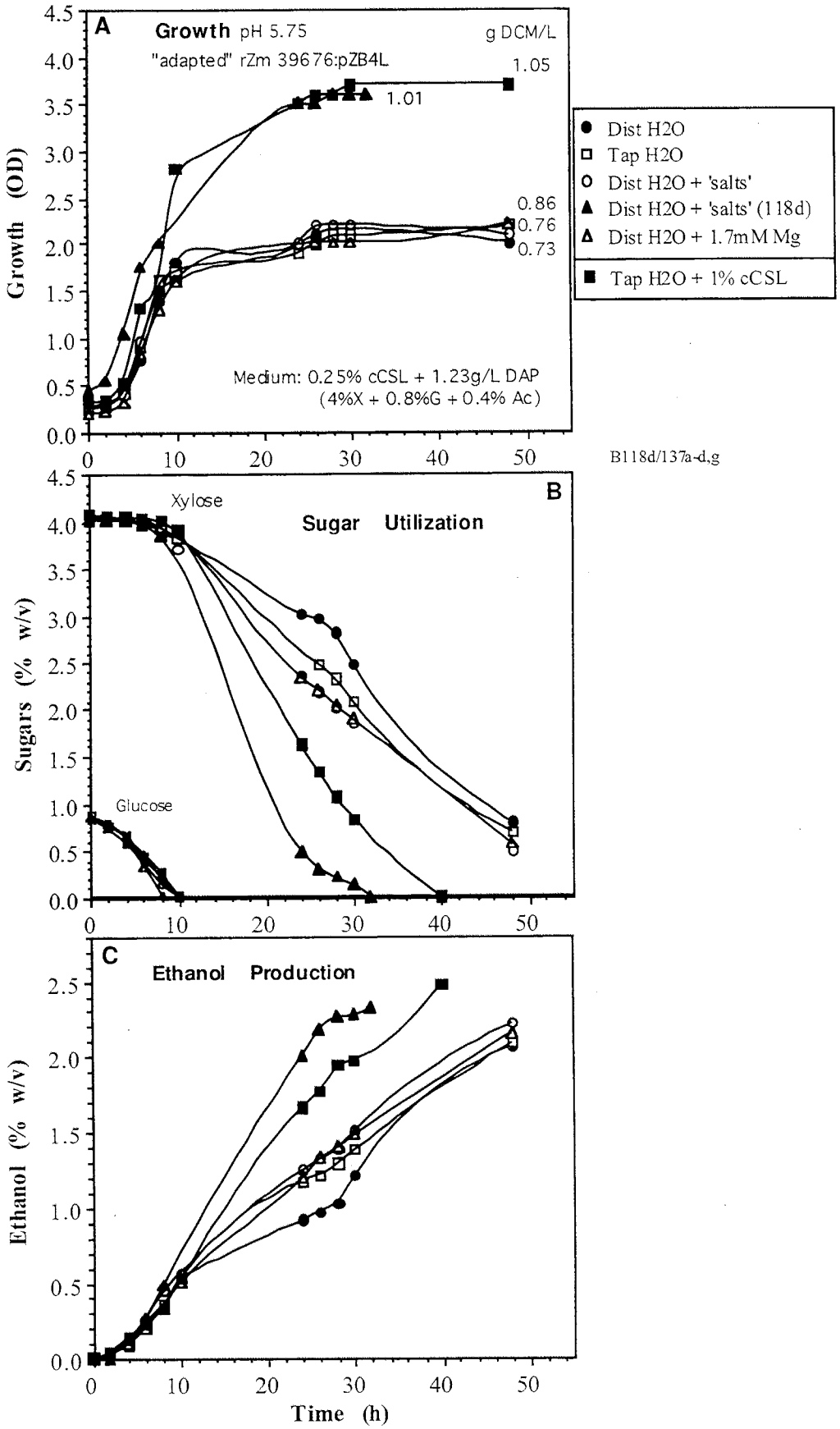


Fig. 3-4 Requirement for salts supplementation with a 0.25% (v/v) cCSL + 0.4% acetic acid medium (**pH 5.75**) and rZm "adapted" 39676:pZB4L [Fig 3, Report #4 - July 23/98]

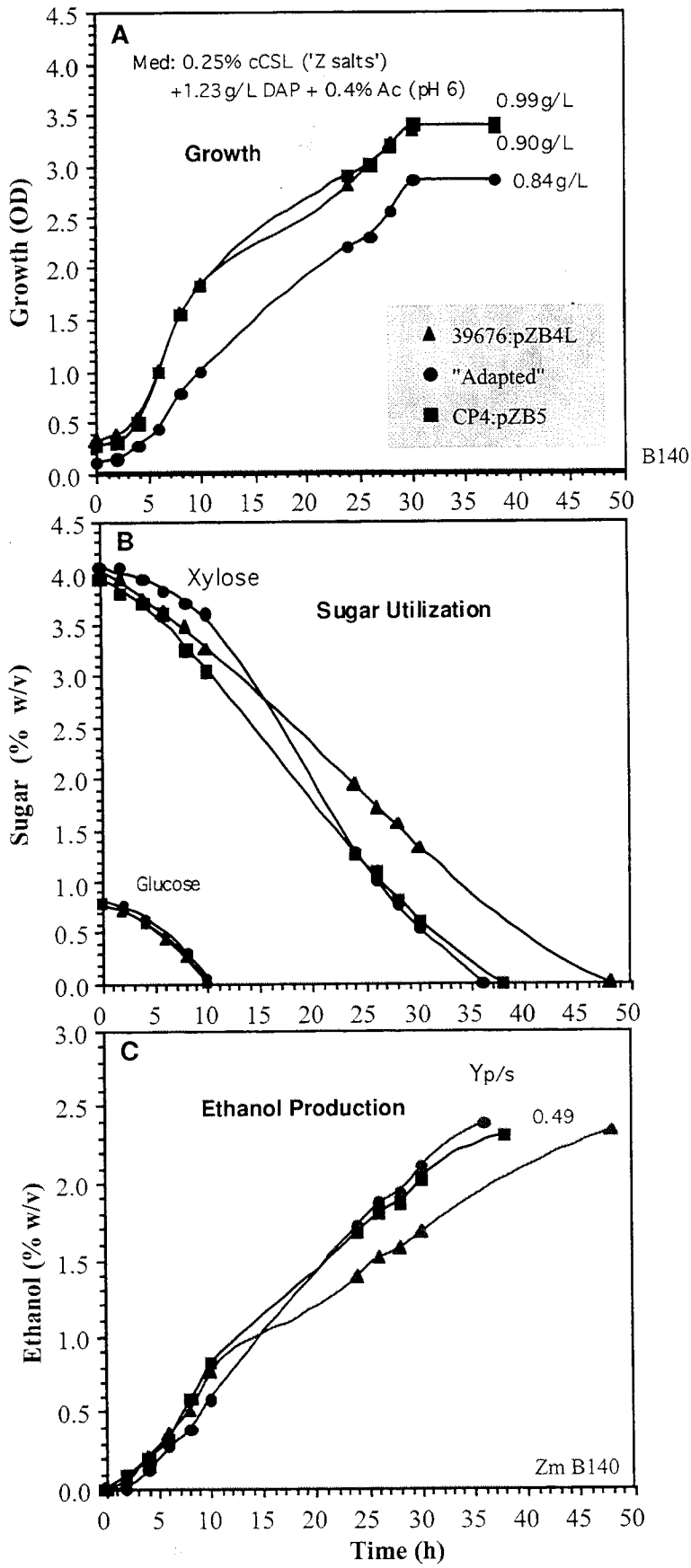


Fig. 3-5 Comparison of 3 strains in similar media: requirement for DAP supplementation with a 0.25% (v/v) cCSL + 0.4% acetic acid medium (pH 6.0)

PART 4

The work described in Part 4 is related to Task #3 of the Statement of Work. The objective was to characterize performance in continuous culture of various feed sugar ratios as a function of dilution rate over the operating range of 0.04 - 0.10 h-1.

This work has already been described in the following contexts:

(i) Technical Progress Report #3 (Figs 2 and 3)
(ii) Technical Progress Report #4 (Fig. 4)
(iii) Technical Progress Report #6 (Fig. 2); #6 (supplemental) (Fig. 1)
(iv) Technical Progress Report #7 (Fig. 2)
(v) NREL Seminar (March 2, 1998) - see Fig. 1 (Appendix J)
(vi) 21st Symposium Paper (1999 - in preparation)

Figure 4-1A shows the time-course of a continuous fermentation with the "adapted" variant in a nutrient-rich medium containing 4% xylose and 0.8% glucose at pH 5.75. The experiment was conducted over a period of 19 days and during that time the dilution rate was increased incrementally from 0.04/h to 0.10/h with the effluent xylose increasing in response to elevated dilution rates (Fig. 4-1A). At no time was glucose detected in the effluent. Figure 4-1B shows the steady-state concentrations of xylose and ethanol as a function of dilution rate.

Previously we published the results of similar experiment using the non-adapted culture (39676:pZB4L) and for comparison purposes Fig 4-2 shows the results of the experiments with both strains superimposed on the same graph. Similar performance for the two strains in continuous cofermentation was expected since the medium did not contain any potentially inhibitory substances (ie. no advantage for the adapted variant). From Fig. 4-2 it would appear that, under this condition (ie. no acetic acid in the medium), the parent strain is superior to the "adapted" strain since the ethanol concentration is maintained higher at the higher dilution rates. However, this conclusion can be questioned in the light of the experiments with 39676:pZB4L that we reported previously (see C60 and C62 in Fig D-20 of Final Report for Phase III, 1997).

Figure 4-3 shows the time-course of a chemostat culture of the "adapted" recombinant using a CSL-based pure sugar synthetic prehydrolysate medium. The difference between the experiments represented in Figs 4-1 and 4-3 is the composition of the medium. The steady-state concentrations

of cell mass and effluent xylose shown in Fig. 4-4 reveals that at a level of 1% (v/v), the clarified CSL (and Z salts) provide sufficient nutrients for the adapted strain to perform optimally. The CSL-based medium produced a slight elevation of the maintenance energy co-efficient from 0.12 to 0.42g sugar/g cell/h (Fig 4-5); however, this is not judged to be significant and may relate to the previously observed tendency of CSL to cause uncoupling.

Figure 4-7 shows the time-course of a chemostat culture of the "adapted" recombinant using a CSL-based pure sugar synthetic prehydrolysate medium containing 3% xylose and 1.8% glucose. The typical ratio of xylose to glucose used in the majority of our synthetic prehydrolysate media has been 5 to 1 (ie. 4% xylose + 0.8% glucose). Therefore, this revised medium with the same total sugar concentration represents a X:G ratio of 1.67 to 1. Batch fermentation studies showed the benefit of increasing the glucose concentration - mainly due to an increase in cell mass. The steady-state concentrations of cell mass and effluent xylose for the 3%X + 1.8%G medium are shown in Fig. 4-7. The difference between Figs 4-4 and 4-7 is simply the composition of the medium with respect to the X:G ratio. Fig 4-8 is a plot of the specific rate of sugar utilization versus D and shows that this alteration to the medium has little affect on either the maximum growth yield or the maintenance energy coefficient. Figure 4-9 shows the time-course for a chemostat experiment with he "adapted" strain in which the nutrient-rich ZM medium contained 4% xylose and 1.4% glucose. In this case the total sugar concentration was increased from 4.8% to 5.4% and the X:G ratio was 2.86 to 1. It was expected that the increased amount of glucose would promote a higher cell mass concentration and this would result in lower effluent xylose levels; however, this was not the case and, despite an attempt at remediation by turning off the feed pump, the xylose concentration was as high or higher than when the medium contained only 0.8% glucose (see Fig. 4-1).

Continuous fermentation of equal amounts of xylose and glucose

2.5% mixture with "adapted" strain

Figure 4-10 shows the time-course of continuous fermentations with the "adapted" recombinant using either a nutrient-rich ZM medium (panel A) or a 1%-CSL medium (panel B) containing 2.5% xylose and 2.5% glucose at pH 5.75. The level of xylose was lower with the ZM medium and this observation was unexpected based on the behaviour in the standard media with 4% xylose and

0.8% glucose (see Fig 4-1 and Fig 4-3). The data from these two experiments were combined and used to formulate the plot shown in Figure 4-11 of the steady-state levels of xylose and cell mass and a function of dilution rate. This information was also used to produce the plots of the specific rate of sugar utilization and specific productivity versus dilution rate that are shown in Fig. 4-12. We noted that with this medium the maintenance energy co-efficient was approx. doubled from 0.5 to about 1 g sugar/g cell/h.

4% mixture with CP4:pZB5

Figure 4-13A shows the time-course of continuous fermentations with CP4:pZB5 using a nutrient-rich ZM medium containing 4% xylose and 4% glucose at pH 5.75. Despite the fact that the system was started at a lower than usual dilution rate of 0.025/h, the xylose level climbed rapidly to a level of about 15g/L within only a few days of the flow being initiated (Fig 4-13A). The xylose level fell to about 7 g/L when the pump was stopped at day 17, but when it was turned on again 48h later, the xylose level increased back to the same value prior to the pumped being shut off (Fig 4-13A). Fig. 4-13B is a plot of the specific rate of sugar utilization and specific productivity versus dilution rate. The maintenance energy co-efficient is close to zero, but the max. growth yield (1/slope) is surprising reduced relative to the standard medium with 4% xylose and only 0.8% glucose. We noted that in their paper presented at the 20th Symposium, Joachimsthal *et al.* quoted values for this same recombinant under similar conditions of 0.08 g DCM.g sugar and 2.5 g sugar/g cell/h g for the max growth yield and maintenance energy, respectively. The pH in their experiment was 5.0 and the D range was extended, but otherwise the conditions were identical. At the higher D values, most of the xylose was not utilized and this have contributed to the shift in their extrapolation to these values for growth yield and maintenance since they are more similar to values expected with *Zymomonas* when glucose is the sole sugar.

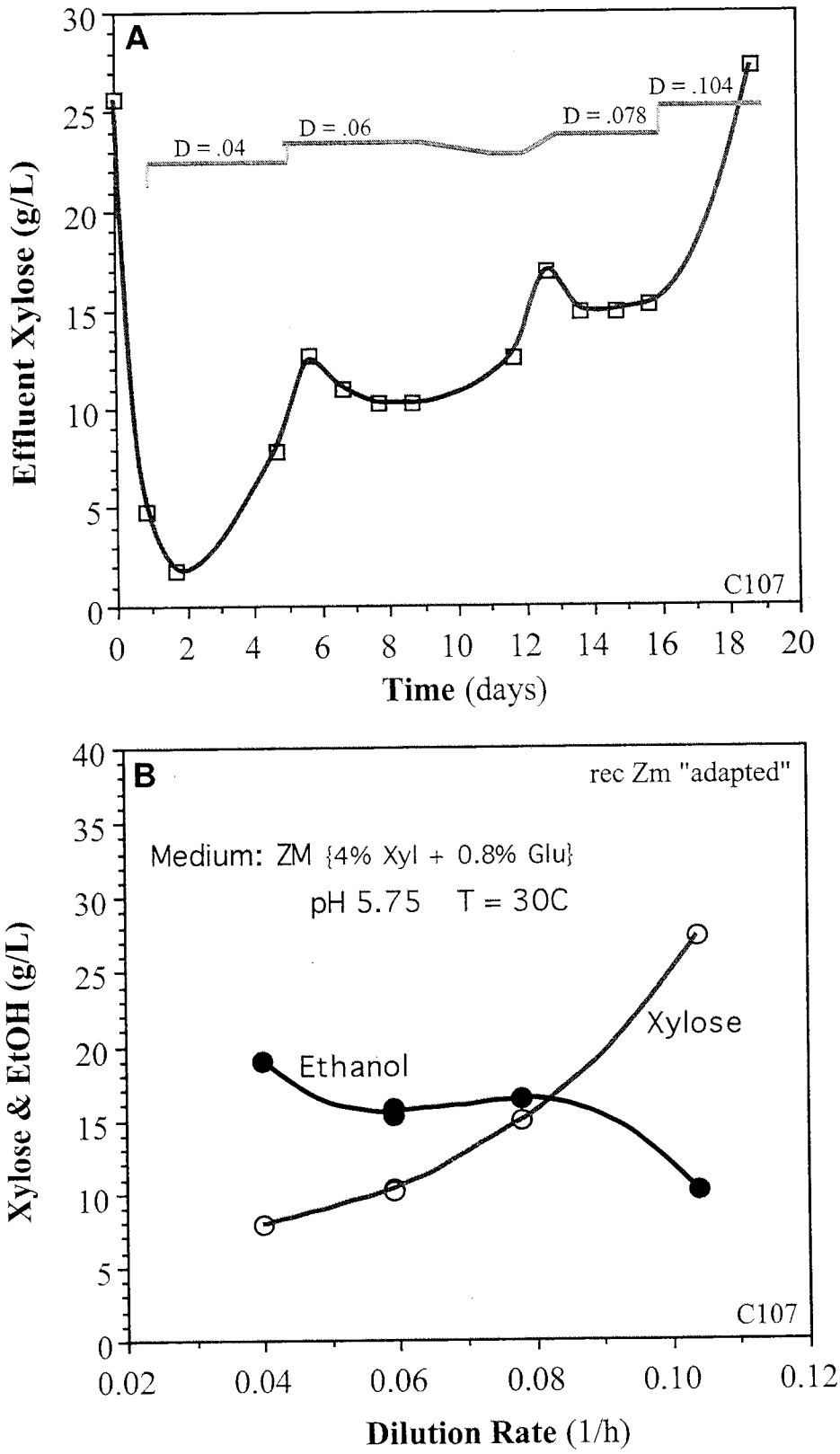


Fig. 4-1 Chemostat culture of rZm "adapted" 39676:pZB4L. The nutrient-rich ZM medium contained 4% xylose + 0.8% glucose. The pH was controlled at 5.75

(ref: Appendix J - NREL Seminar - March 2, 1998)

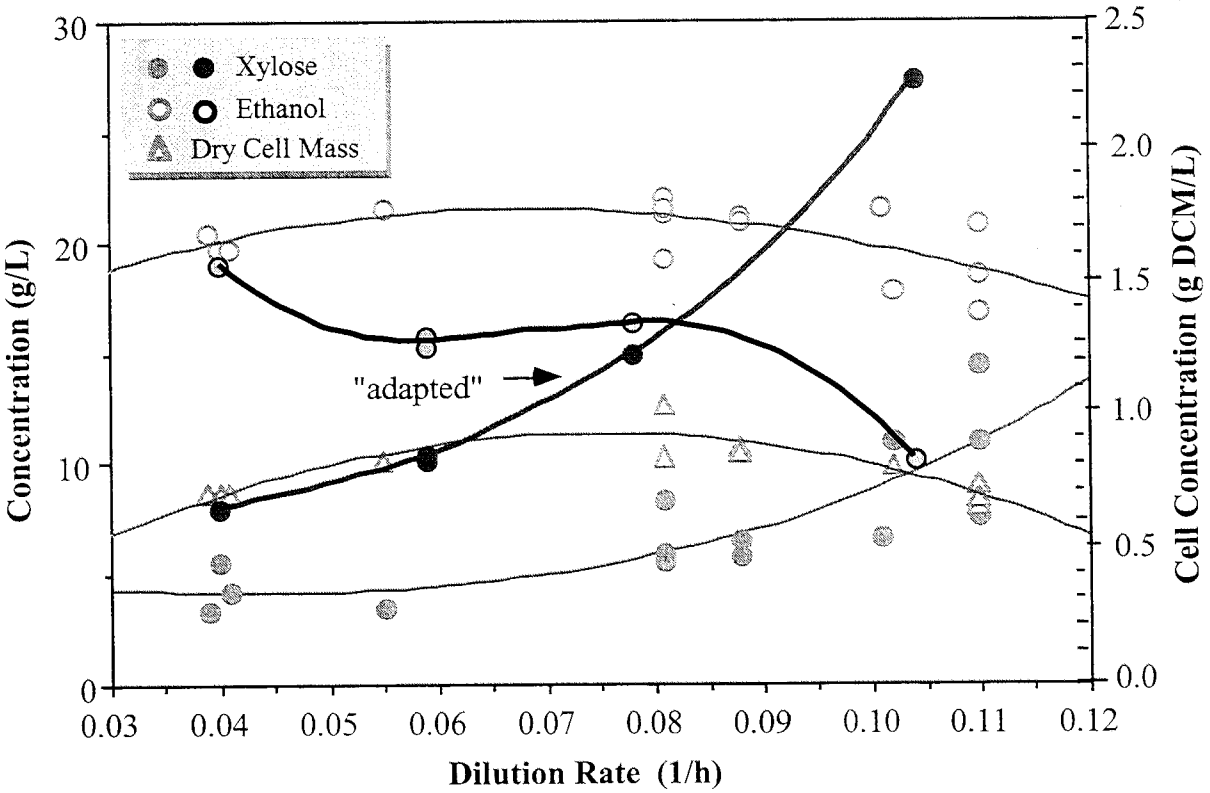


Fig. 4-2 Chemostat culture of rZm "adapted" 39676:pZB4L

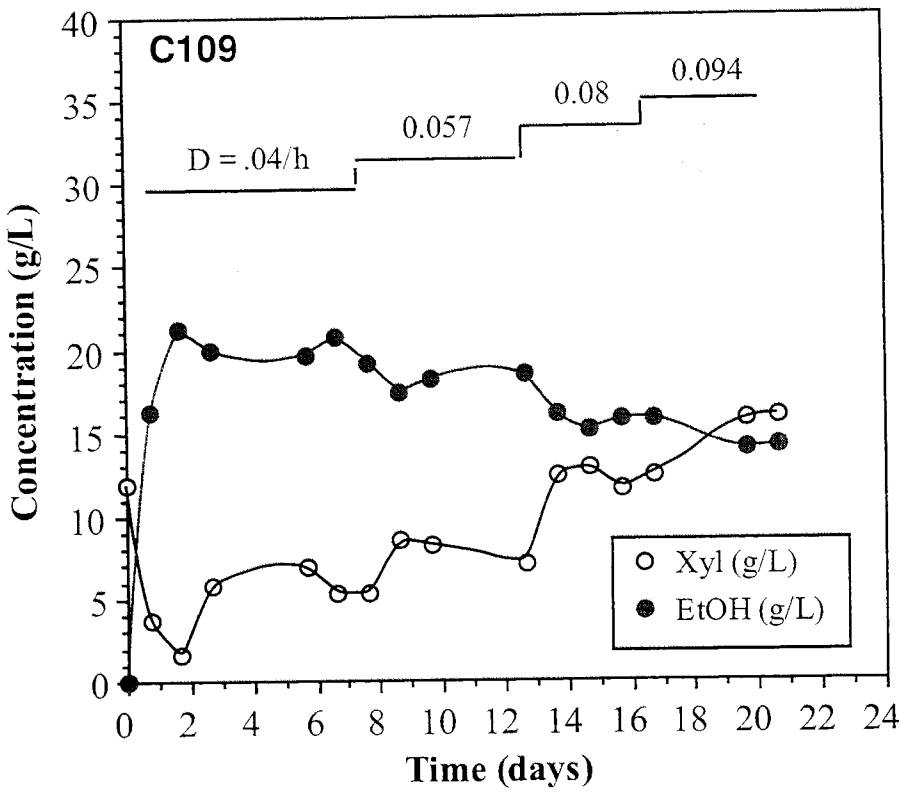


Fig. 4-3 Continuous fermentation of "adapted" 39676:pZB4L with 4% xylose + 0.8% glucose (1% v/v cCSL + Z salts) at pH 5.75. Expt C109 "Z-salts" are defined in *Materials & Methods* Compare to Fig 4-1 with ZM medium (Expt C107)

(ref: Fig. 1, Prog Report # 6 - Supplemental)

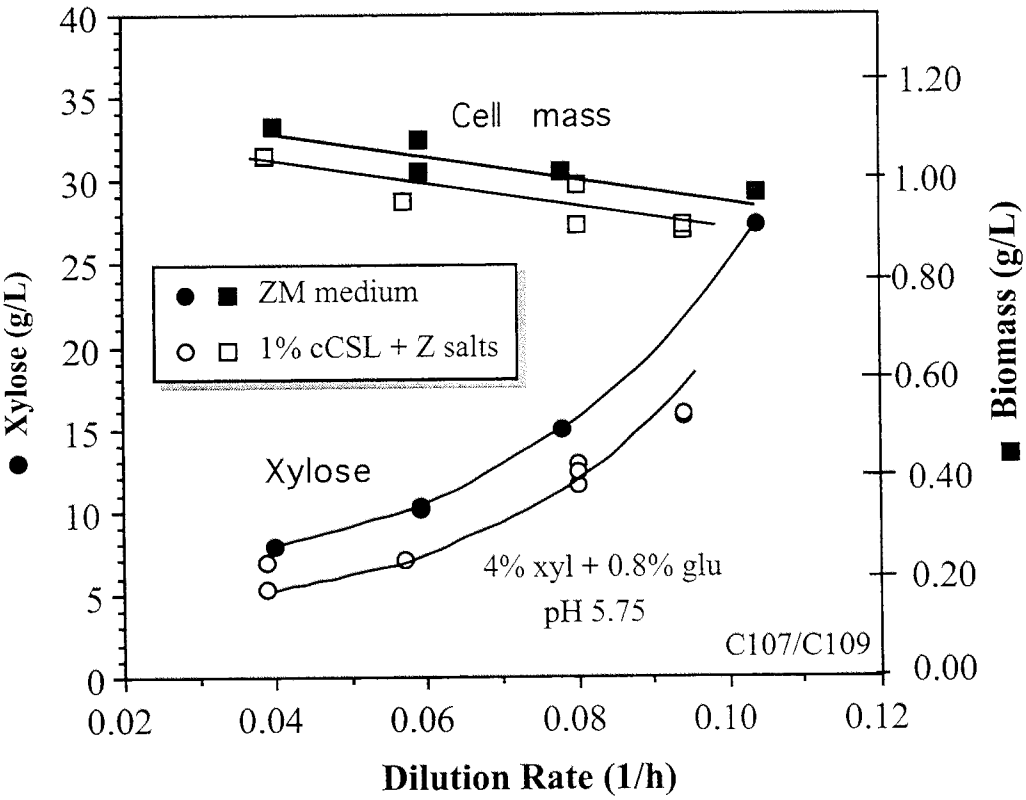


Fig 4 - 4 Continuous fermentation of "adapted" 39676:pZB4L with 4% xylose + 0.8% glucose (ZM or 1% w/v ccSL + Z salts) at pH 5.75. Expts C107 and C109

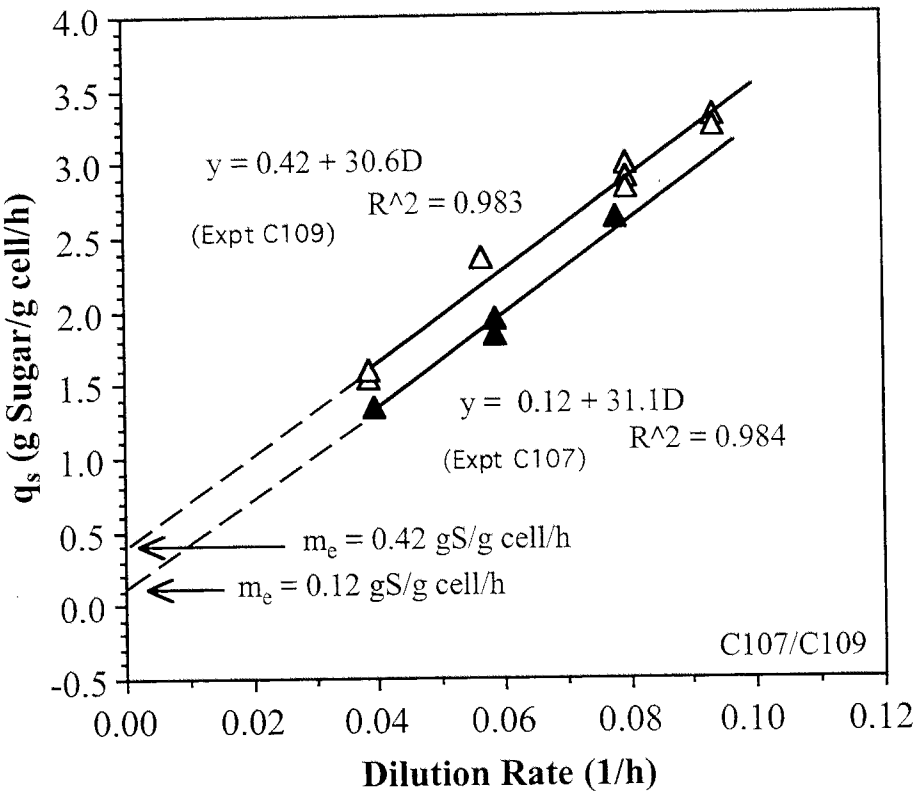


Fig. 4-5 Continuous fermentation of "adapted" 39676:pZB4L with 4% xylose + 0.8% glucose (ZM or 1% cCSL + Z salts) at pH 5.75. Expts C107 and C109

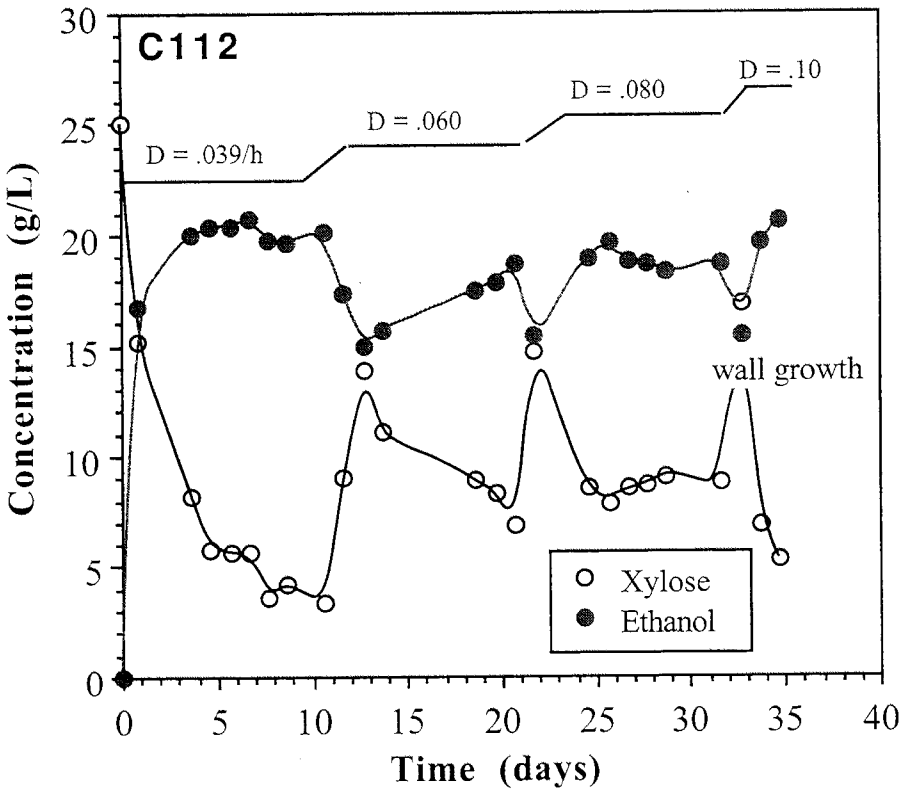


Fig. 4-6 Continuous fermentation of "adapted" 39676:pZB4L with 3% xylose + 1.8% glucose (1% cCSL + Z salts) at pH 5.75. Expt C112

(ref: Fig. 4, Prog Report #4)

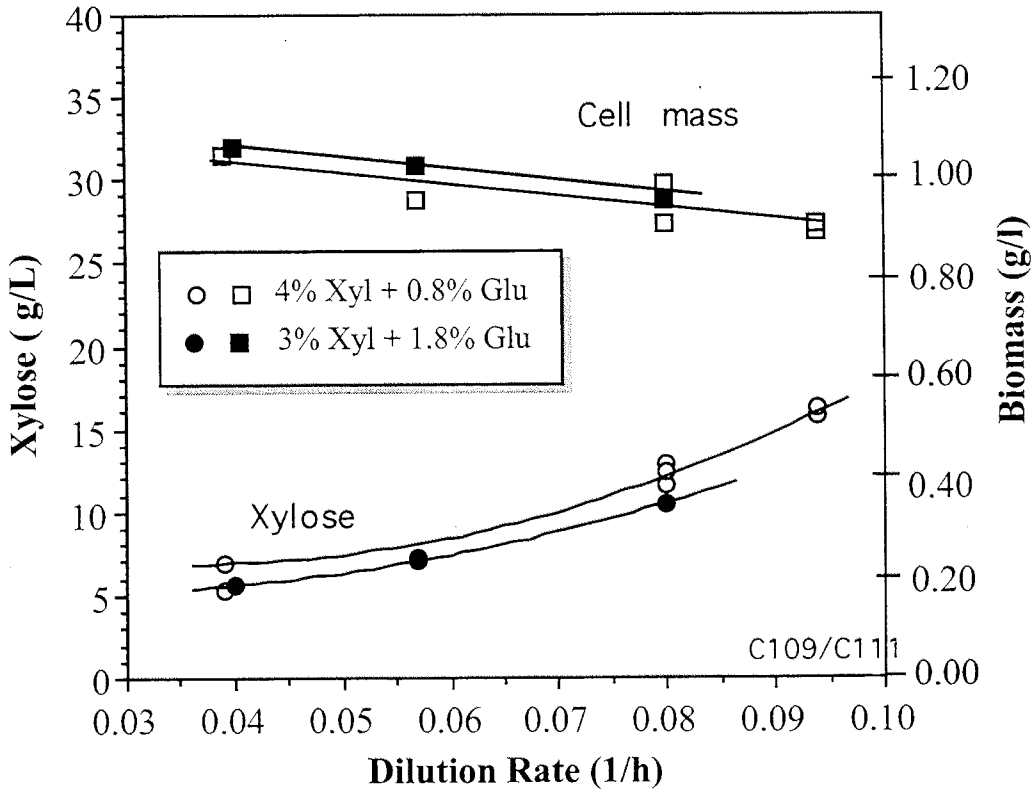


Fig. 4-7 Continuous fermentation of "adapted" 39676:pZB4L with either 4% xlyose + 0.8% glucose or 3% xylose + 1.8% glucose (1% cCSL + Z salts) at pH 5.75. Expts C109 and C111

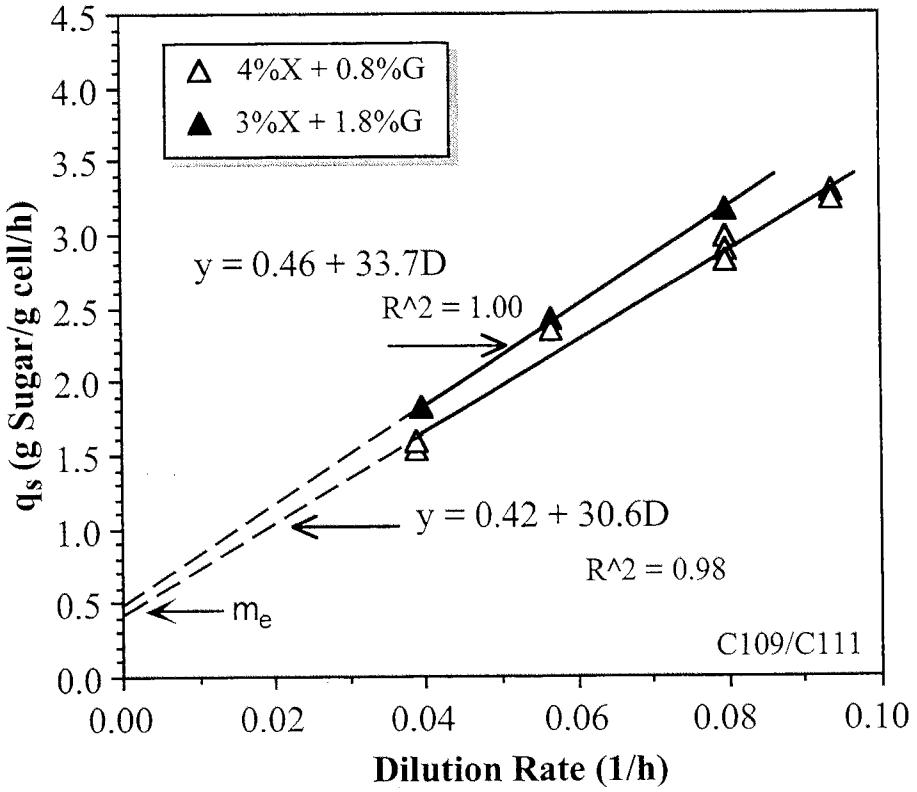


Fig. 4-8 Continuous fermentation of "adapted" 39676:pZB4L with either 4% xlyose + 0.8% glucose or 3% xylose + 1.8% glucose (1% cCSL + Z salts) at pH 5.75. Expts C109 and C111

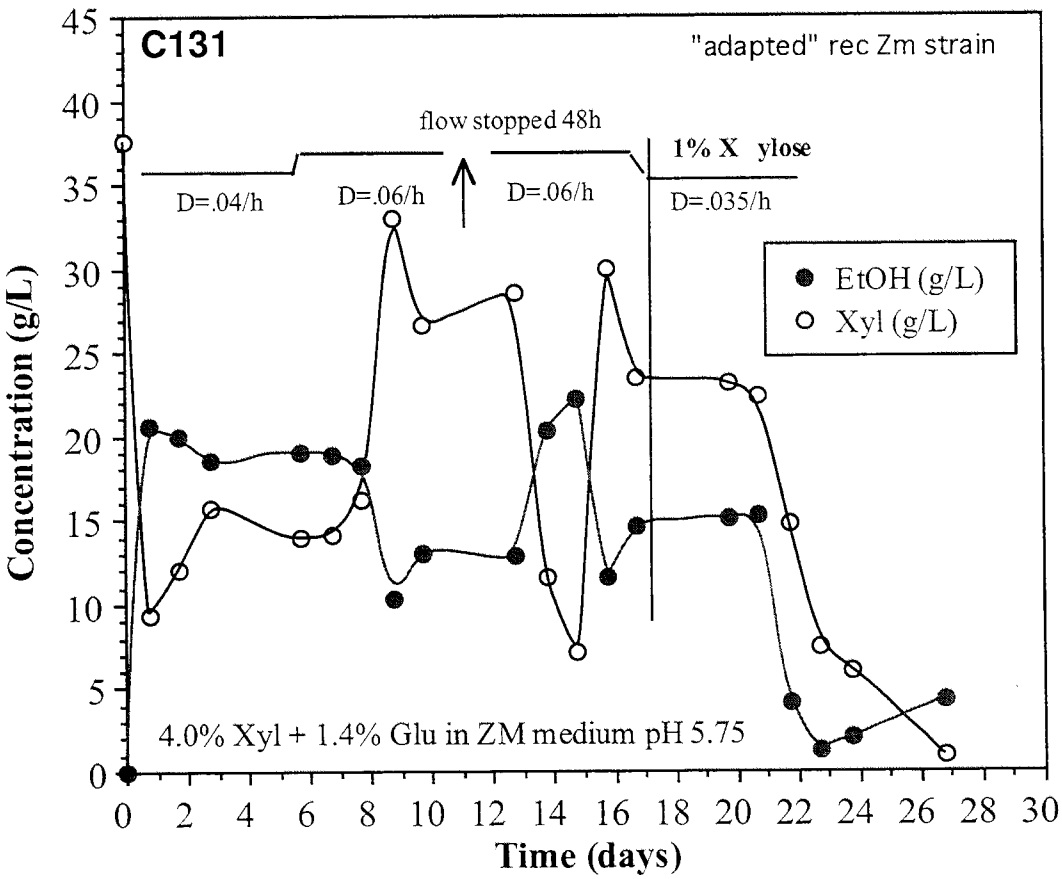
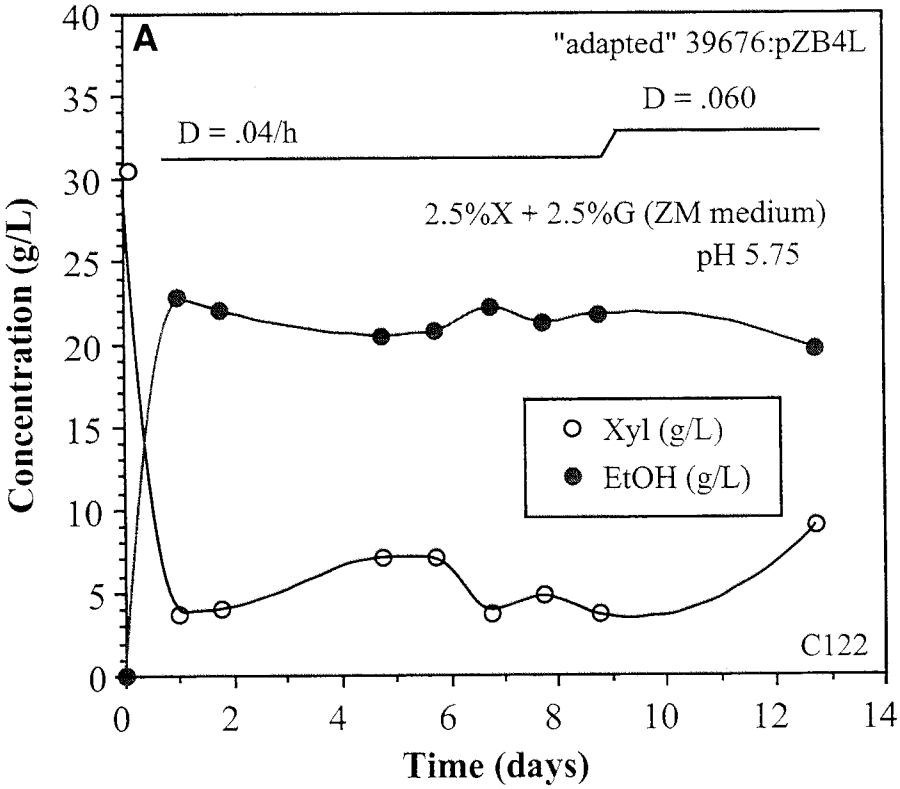


Fig. 4-9 Continuous fermentation of "adapted" 39676:pZB4L with 4% xlyose + 1.4% glucose in ZM medium at pH 5.75. Expt C131

(ref: Fig. 2, Prog Report #7)



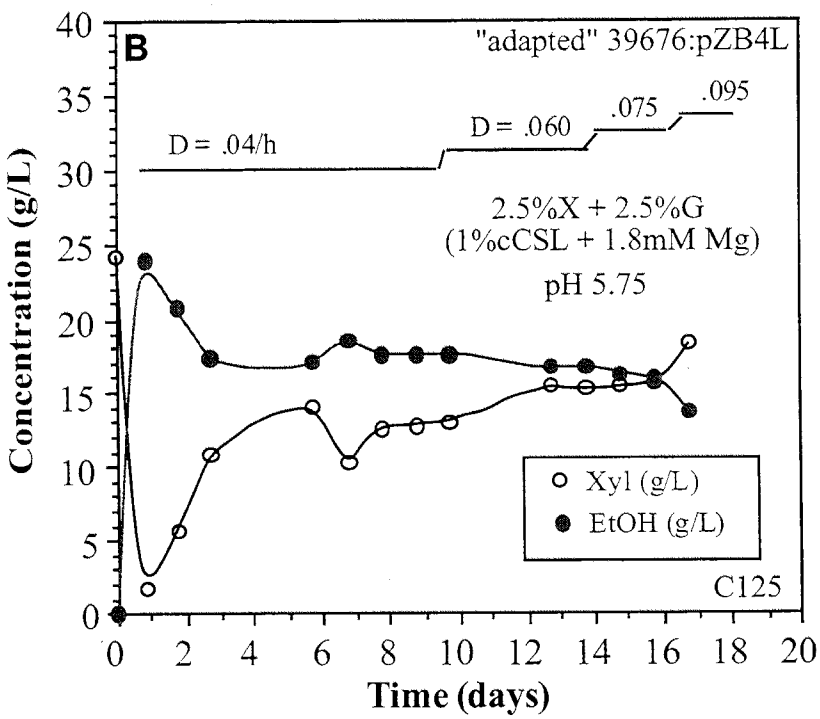


Fig. 4-10 Continuous fermentation of "adapted" 39676:pZB4L with 2.5% xlyose + 2.5% glucose (**A**) in ZM medium, (**B**) in 1% cCSL + 1.8mM Mg, at pH 5.75. Expts C122 and C125

(ref: Figs. 1 and 2, Prog Report #6)

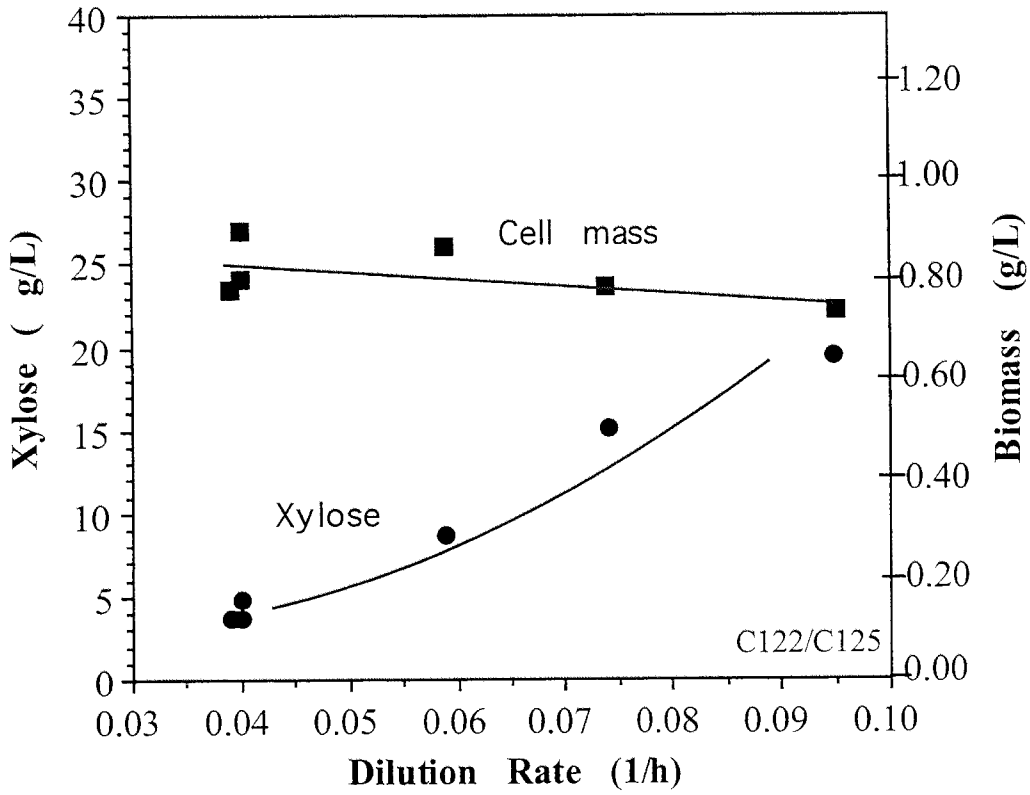


Fig. 4-11 Continuous fermentation of "adapted" 39676:pZB4L with 2.5% xlyose + 2.5% glucose at pH 5.75. Combined data from expts C122 and C125

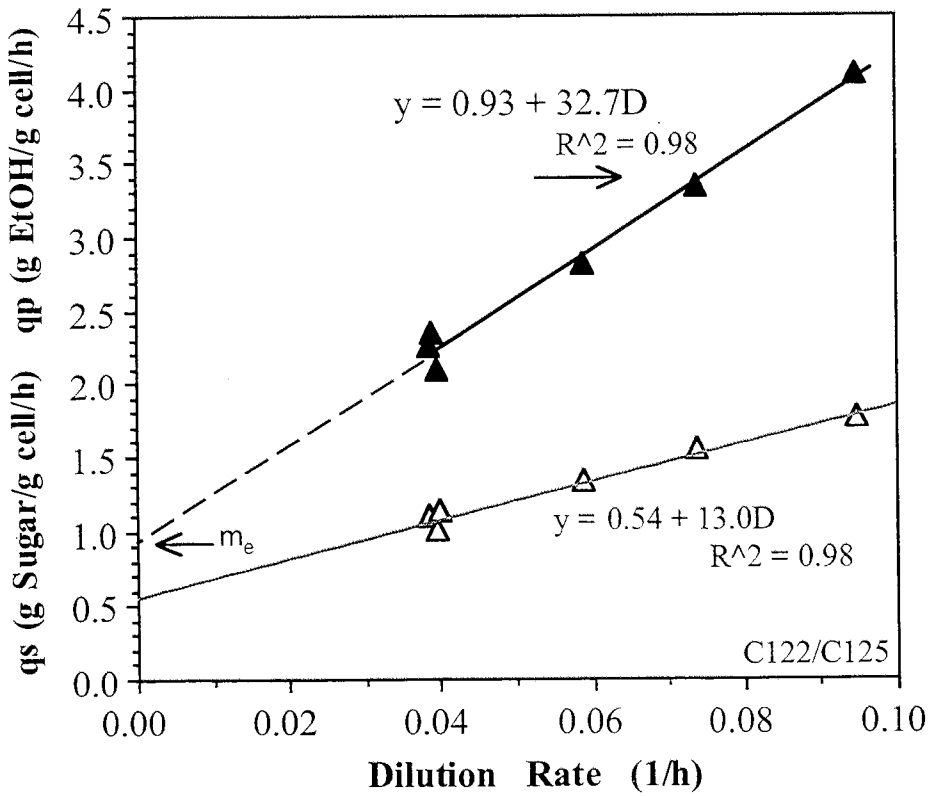
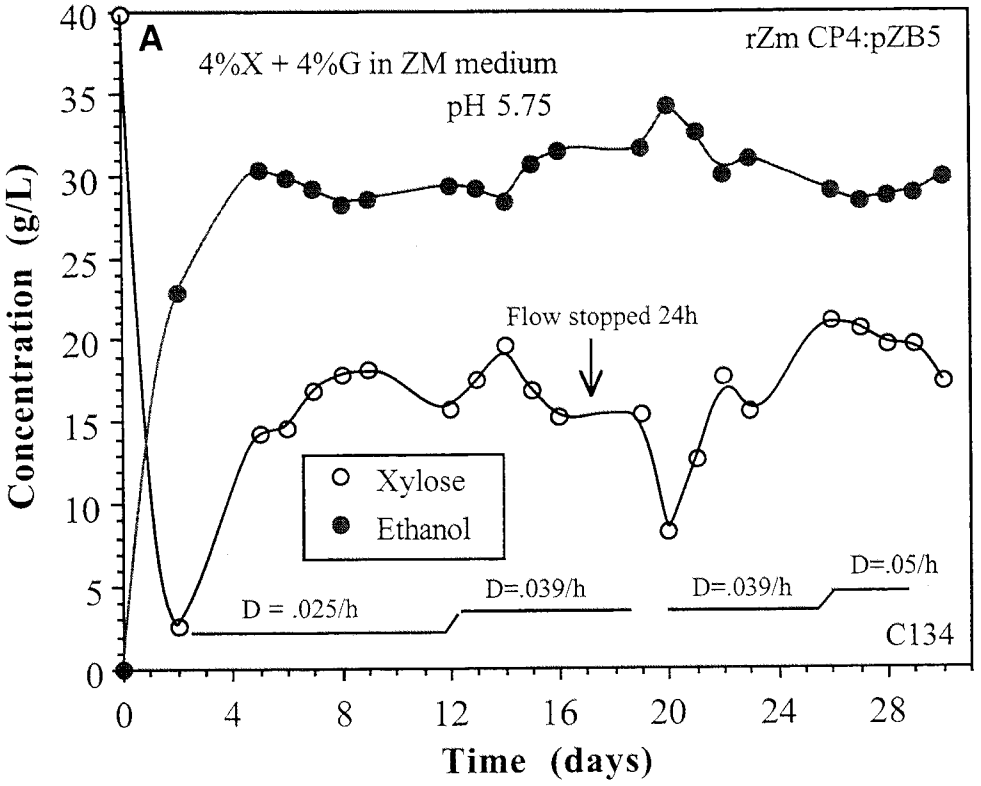


Fig. 4-12 Continuous fermentation of "adapted" 39676:pZB4L with 2.5% xlyose + 2.5% glucose at pH 5.75. Combined data from expts C122 and C125



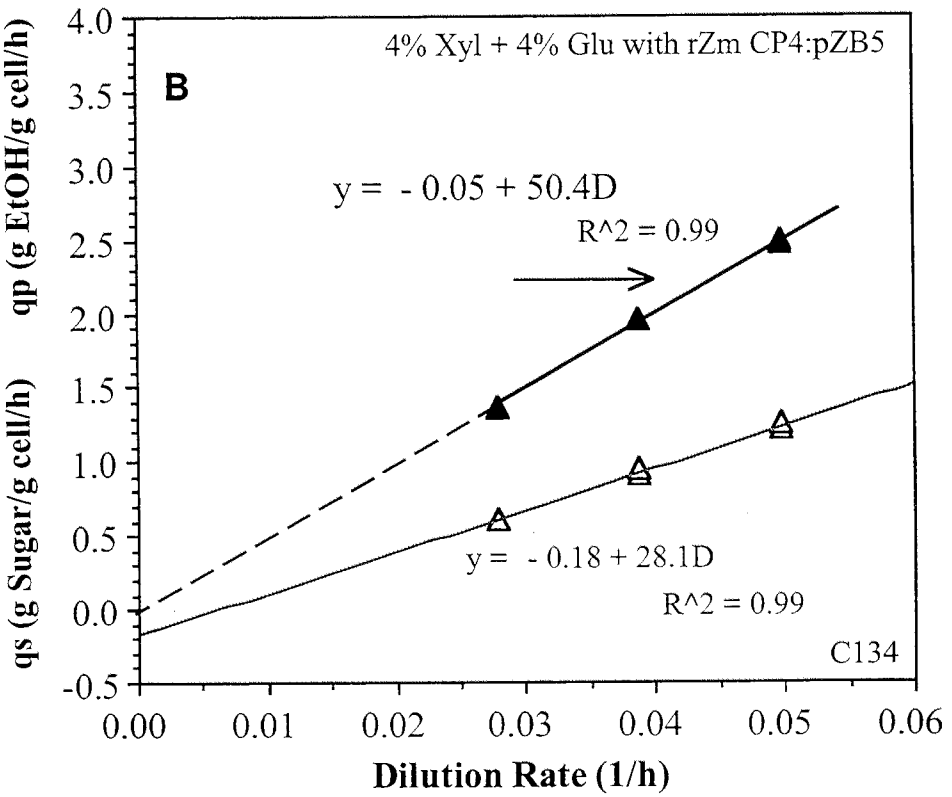


Fig. 4-13 Continuous fermentation of rec Zm CP4:pZB5 with 4% xlyose + 4% glucose at pH 5.75. Expt C134

PART 5

The work described in Part 5 is related to Task #4 of the Statement of Work. The objective was to assess the effect of acetic acid on chemostat performance using two feed sugar and two acetic acid concentrations.

This work has already been described in the following contexts:

(i) Technical Progress Report #4 (Figs. 5 - 9, 11)
(ii) Technical Progress Report #5 (Fig. 6)
(iii) Technical Progress Report #6 (Fig.3)
(iv) 21st Symposium Paper (in preparation)

Experimental design

All of the continuous fermentation experiments described in this Part of the Report are with the "adapted" recombinant (derived from 39676:pZB4L). In all cases the pH was controlled at 5.75 and the temperature was maintained at 30°C. In all cases the medium was 1% (v/v) clarified CSL (cCSL); however, sometimes tap water (TW) was used and in other instances distilled water with either a salts cocktail ("Z salts" - see *Materials & Methods*; Part 3) or 1.8mM $MgSO_4$ were added. Two different sugar combinations were used with a total sugar concentration of 4.8% (w/v): either 4% xylose + 0.8% glucose or 3% xylose + 1.8% glucose. The amount of acetic acid was varied within the range 0 - 0.4% (w/v) except in Expt. C119 (see Fig 5-4) where the initial acetic acid level was 0.8% but this was subsequently reduced to 0.2% after 10 days of continuous operation.

Effect of acetic acid with CSL medium (4% X + 0.8% G) at pH 5.75

Figures 5-1 and 5-2 are time courses for continuous fermentations with the adapted strain and CSL-based media containing 4% xylose and 0.8% glucose with 0.2% and 0.4% (w/v) acetic acid, respectively. Figure 5-3 is a Pirt plot of the specific rate of sugar utilization versus D (steady-state dilution rate) for the expt. represented in Fig. 5-2 with 0.4% acetic acid. For comparison purposes we have also included in Fig. 5-3 data from the expt. C109 using the same medium without added acetic acid. The presence of this amount of acetic acid at pH 5.75 appears to affect the maximum growth yield (inverse slope in plot of q_s vs D) whereas the maintenance energy coefficient (approx. 0.5 g sugar/g cell/h) (y-axis intercept in plot of q_s vs D) is relatively unaffected (Fig. 5-3). The max. growth yield decreased from 0.032 to 0.023 g DCM/g sugar with 0.4% acetic acid in the medium (Table 7).

Effect of acetic acid with CSL medium (3% X + 1.8% G) at pH 5.75

Figures 5-4. 5-5, 5-6A and 5-6B are time courses for continuous fermentations with the adapted strain and CSL-based media containing 3% xylose and 1.8% glucose with either 0.2% or 0.4% (w/v) acetic acid. Figures 5-5 and 5-6 are essentially repeats of the same experiment and are intended to show the variability in the pattern with respect to effluent xylose. Although the inorganic composition of the media in these experiments was intentionally different, it is not believed to be a contributing factor to the observed variability (eg Fig 5-6A and 6B).

Figure 5-7 is a plot of cell mass and effluent xylose concentrations as a function of steady-state dilution rate for the experiments represented in Fig. 5-5 and 5-6A. At pH 5.75, the presence of 0.4% acetic acid in the medium causes a significant reduction in cell mass and at D=0.06/h the effluent xylose is 2-3 times higher with the acetate medium (Fig. 5-7).

Figure 5-8 is a Pirt plot of the specific rate of sugar utilization versus D (steady-state dilution rate) for the expts. represented in Fig. 5-5 and 5-6A with 0.4% acetic acid. For comparison purposes we have also included in Fig. 5-8 data from the expt. C111 using the same medium without added acetic acid. The presence of this amount of acetic acid at pH 5.75 appears to affect primarily the maintenance energy coefficient whereas the maximum growth yield is relatively unaffected (Fig. 5-8). The max. growth yield was about 0.03g DCM/g sugar with or without 0.4% acetic acid in the medium (Table 7). Curiously, the sugar composition of the medium in the absence of any acetic acid appeared to affect the maintenance energy coefficient which was about 0.5 in the 4%X + 0.8%G medium, but close to zero in the 3%X + 1.8%G medium (Table 7). These difference are most likely a reflection of the error in linear regression with data at limited D values.

Effect of 0.4% acetic acid on ethanol productivity at D = 0.04/h (pH 5.75)

Figure 5-9 summarizes the results of this part of the work by showing the effect of 0.4% acetic acid on the volumetric productivity at D = 0.04/h as a function of the medium composition with respect to xylose and glucose. In the absence of acetic acid the volumetric productivity for 4%X + 0.8%G and 3%x + 1.8%G media are 0.78 and 0.82 g ethanol/L/h, respectively. The addition of 0.4% acetic acid causes the volumetric productivity to decrease to 0.67 and 0.74 g ethanol/L.h for these two media, respectively (Fig. 5-9). For each medium, this level of acetic acid (at pH 5.75) causes about 10-15% reduction in productivity and the pattern of effluent xylose is also almost identical (Fig. 5-9)

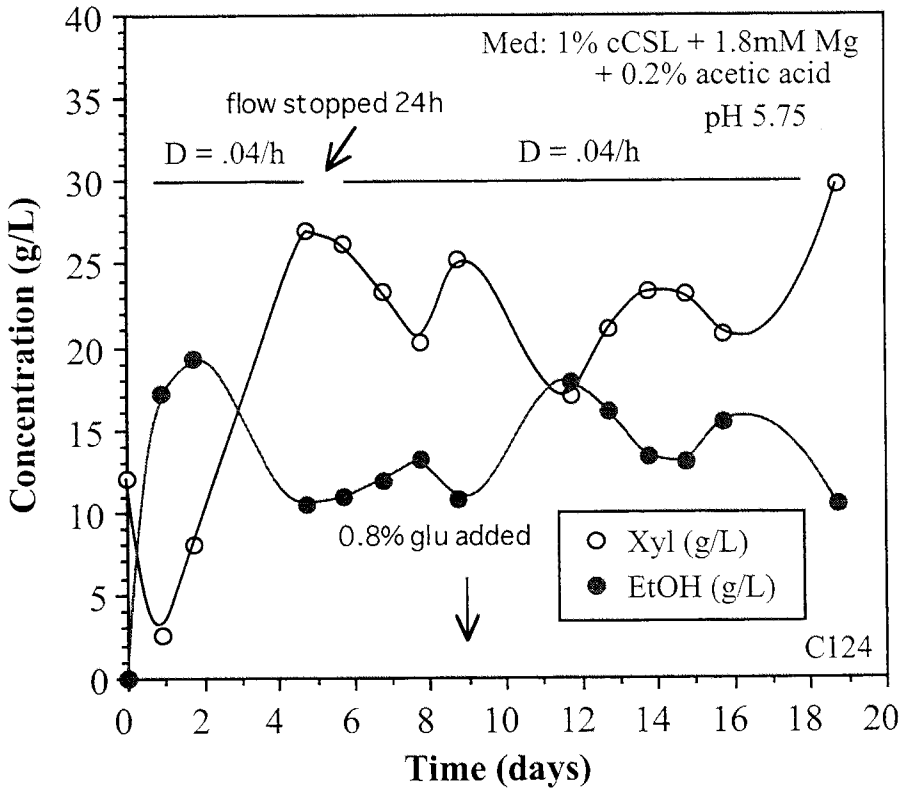


Fig. 5-1 Continuous fermentation of "adapted" 39676:pZB4L with 4% xlyose + 0.8-1.6% glucose and 0.2% (w/v)acetic acid (1% cCSL in dist H_2O + 1.8mM Mg) at pH 5.75. After 9d, 0.8% glucose was added to the feed (see arrow). Expt. C124

(ref: Fig. 3, Prog Rep #6)

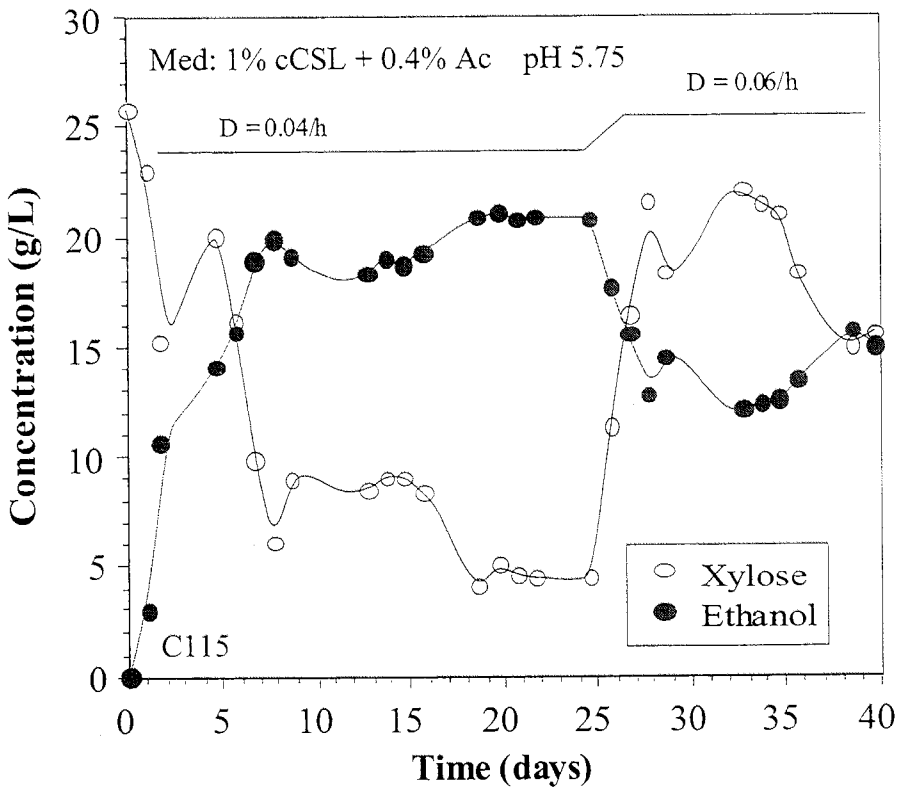


Fig. 5-2 Continuous fermentation of "adapted" 39676:pZB4L with 4% xlyose + 0.8% glucose and 0.4% acetic acid (1% cCSL in TW) at pH 5.75. Expt C115

(ref: Fig. 6, Prog Rep. #4 - July 23/98)

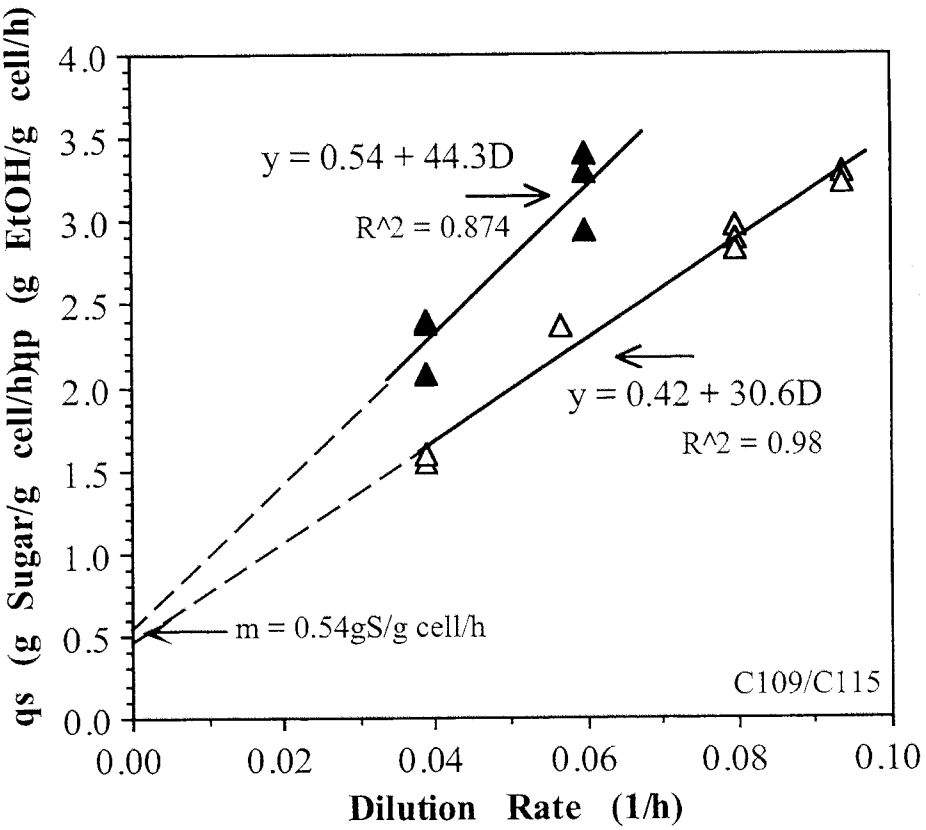


Fig. 5-3 Pirt plot for "adapted" 39676:pZB4L with 4% xlyose + 0.8% glucose -/+ 0.4% (w/v) acetic acid (1% cCSL) at pH 5.75. Expts C109 (CSL in TW) and C115 (CSL in dist H_2O + Z salts) Lines are computer generated by linear regression ; m = maintenance energy coefficient. Parameters are summarized in Table 6

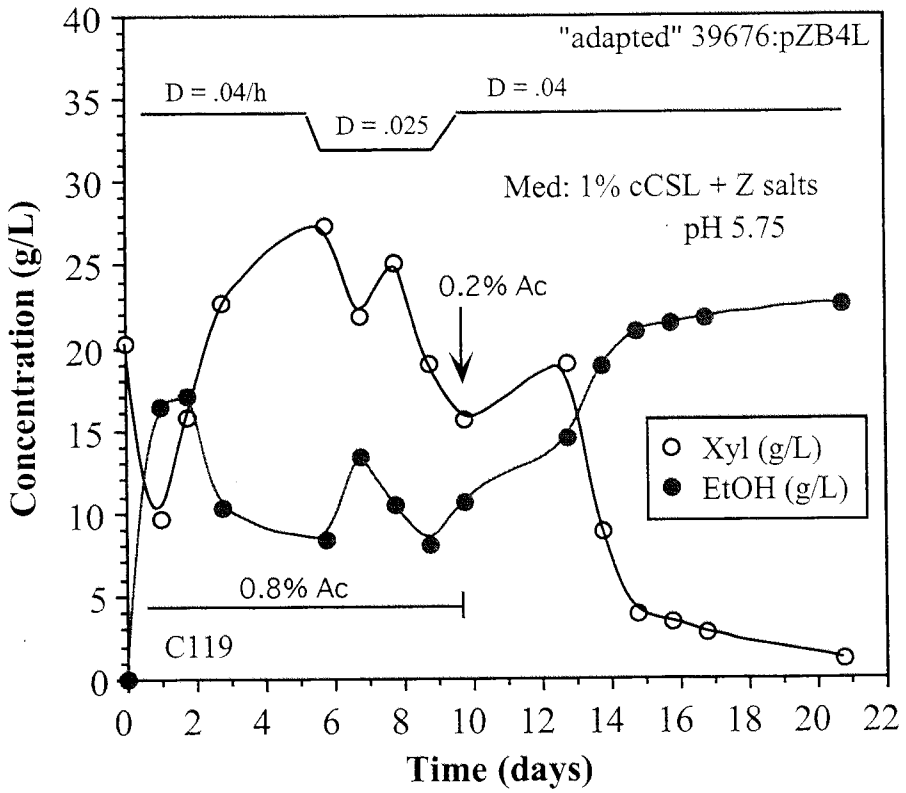


Fig. 5-4 Continuous fermentation of "adapted" 39676:pZB4L with 3% xlyose + 1.8% glucose and acetic acid (1% cCSL in dist H_2O + Z salts) at pH 5.75. After 10d the acetic acid in the feed was lowered from 0.8% to 0.2% (see arrow). Expt C119

(ref: Fig. 6, Prog Report #5)

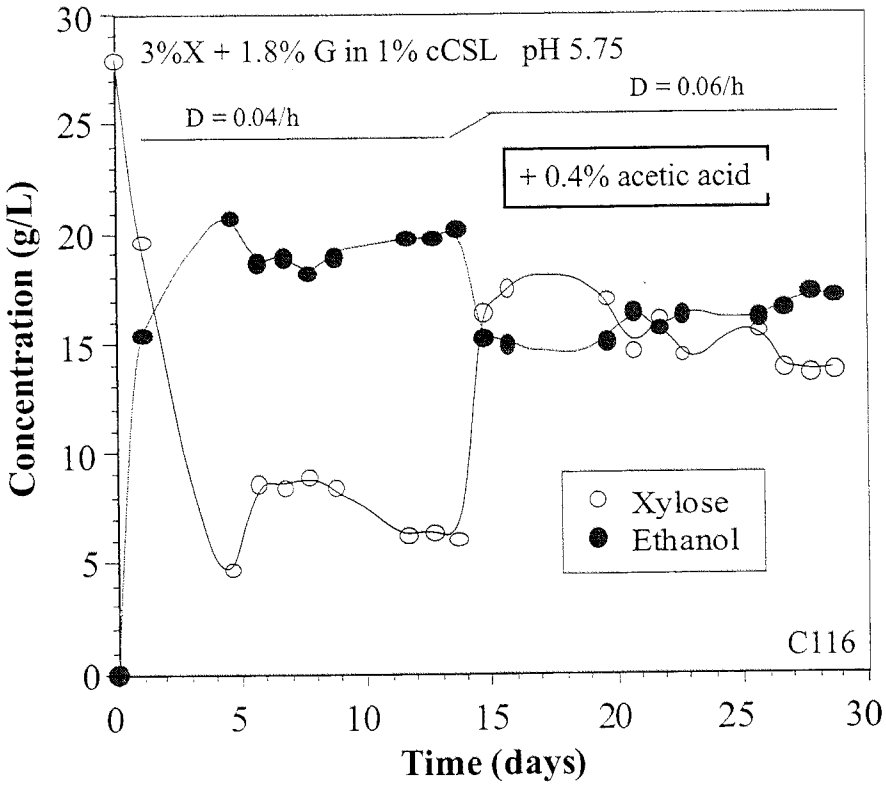


Fig. 5-5 Continuous fermentation of "adapted" 39676:pZB4L with 3% xlyose + 1.8% glucose and 0.4% (w/v) acetic acid (1% cCSL in TW) at pH 5.75. Expt C116

(ref: Fig. 8, Prog Report #4 - July 23/98)

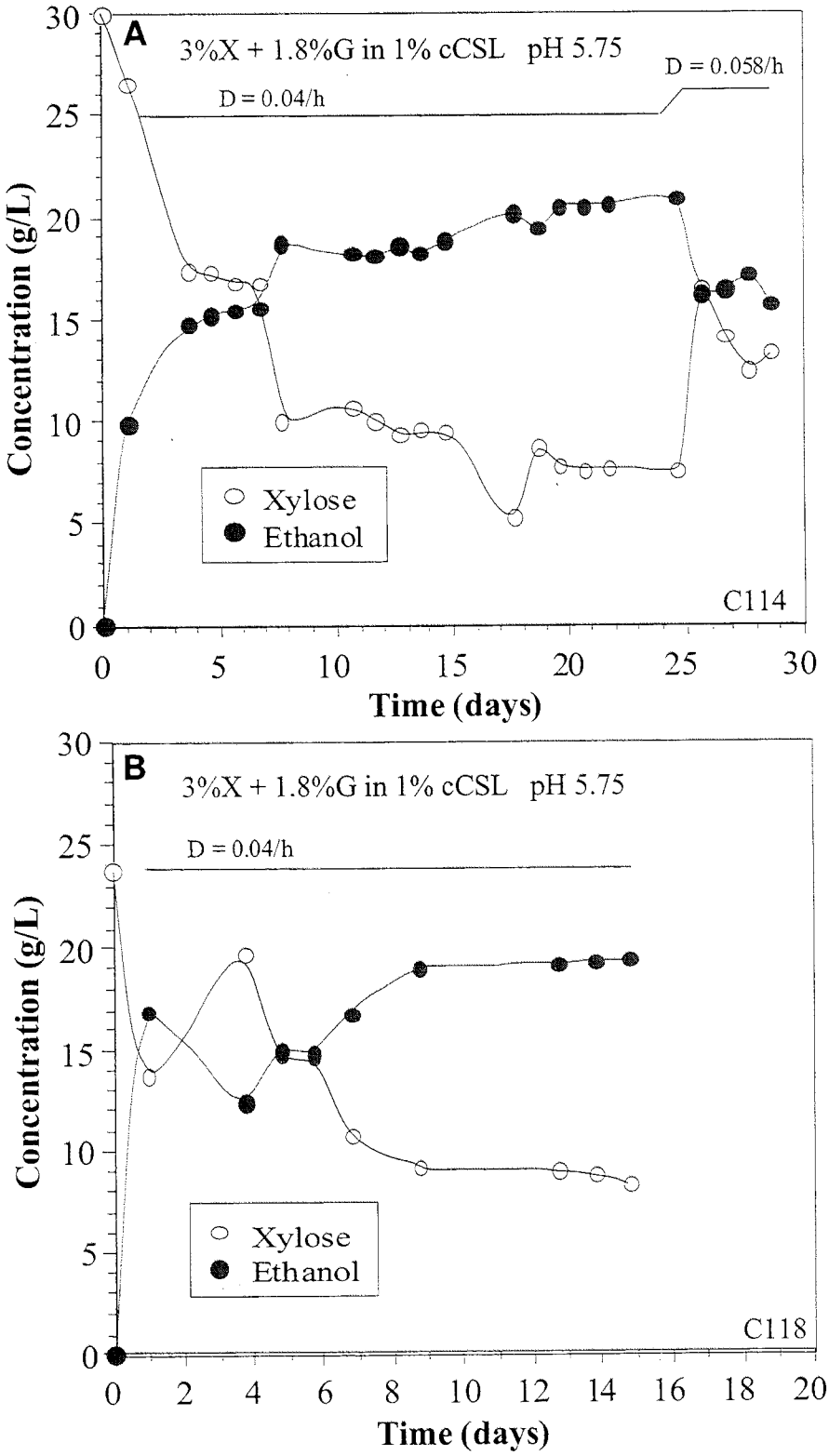


Fig. 5-6 Continuous fermentation of "adapted" 39676:pZB4L with 3% xlyose + 1.8% glucose and 0.4% (w/v) acetic acid (1% cCSL) at pH 5.75.
Expts C114 (CSL in TW) and C118 (CSL in TW + "Z salts")

(ref: Figs. 5 and 9, Prog Report #4)

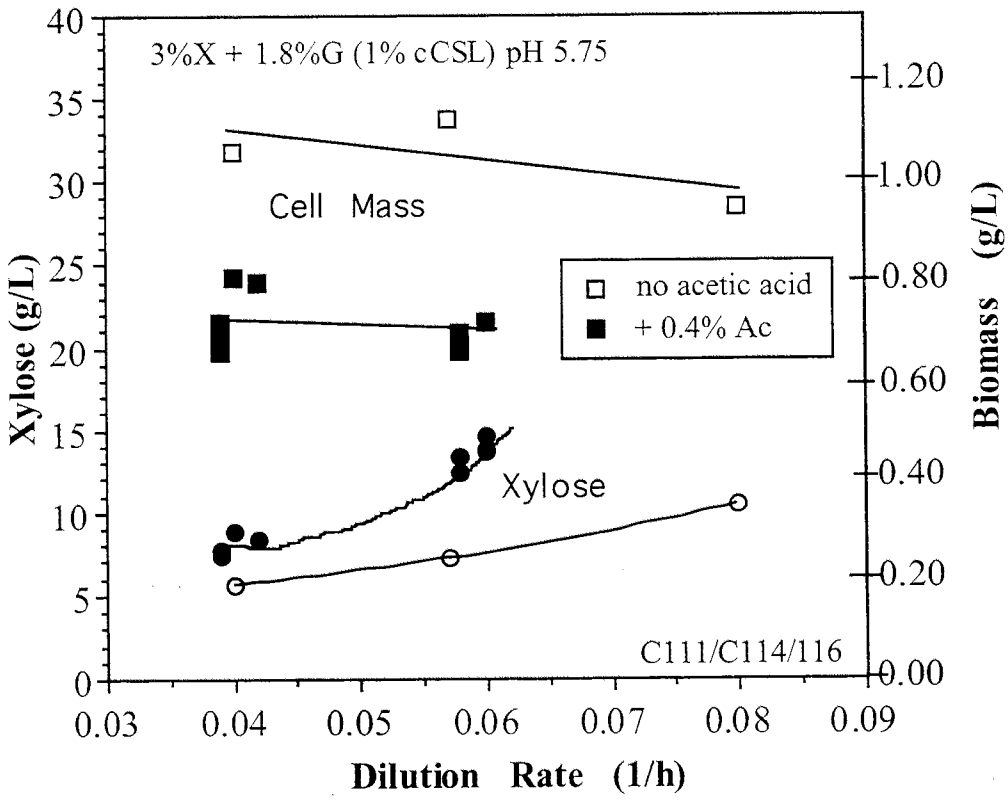


Fig. 5-7 Continuous fermentation of "adapted" 39676:pZB4L with 3% xlyose + 1.8% glucose -/+ 0.4% acetic acid (1% cCSL) at pH 5.75. Steady-state cell mass and effluent xylose concentrations as a function of dilution rate. Expts. C111, C114 and C116 Symbols: open squares are without acetic acid and filled squares are with 0.4% (w/v) acetic acid in the feed.

(ref: Fig. 11, Prog Report # 4)

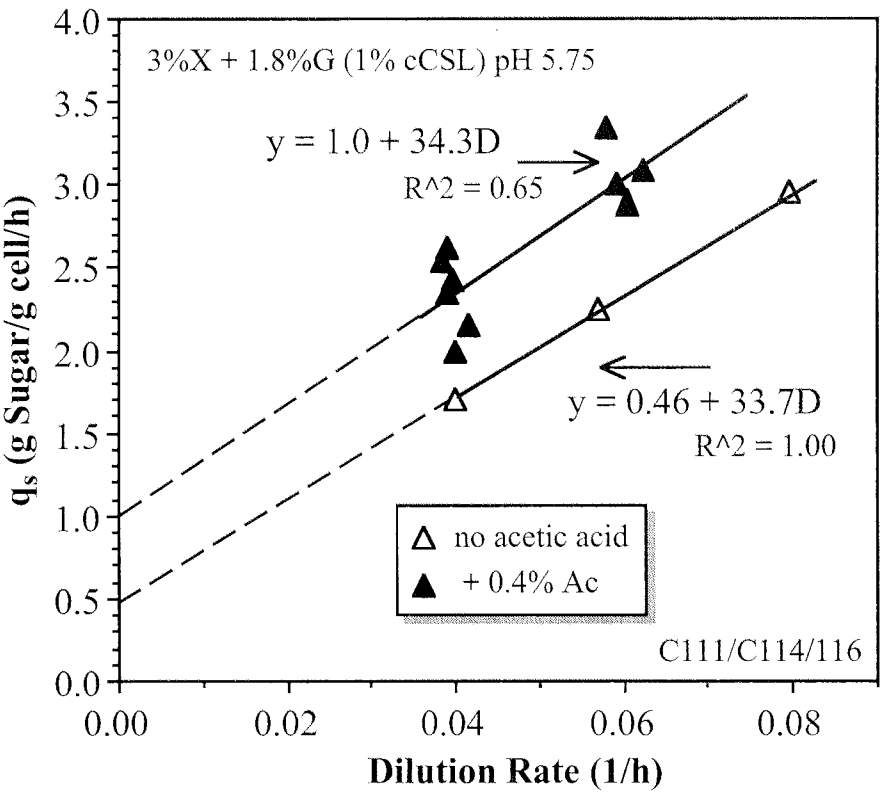


Fig 5 - 8 Pirt plot for "adapted" "adapted" 39676:pZB4L with 3% xlyose + 1.8% glucose -/+ 0.4% (w/v) acetic acid (1% cCSL) at pH 5.75. Expts C111, C114 and C116 Lines are computer generated by lineare regression. Parameters are summarized in Table 7

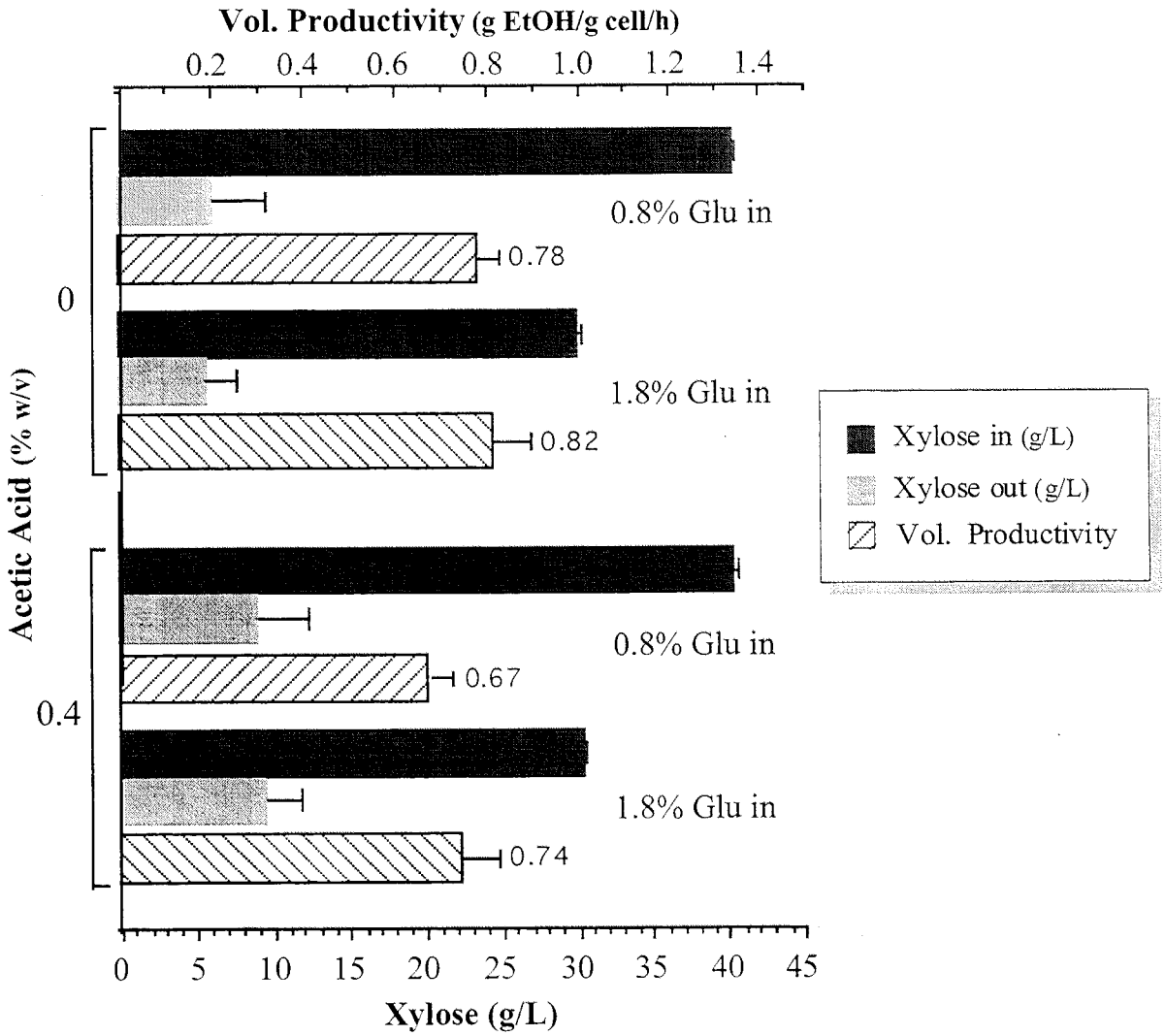


Fig. 5-9 Effect of 0.4% (w/v) acetic acid on "adapted" rZm 39676:pZB4L in continuous fermentations at D = 0.04/h and pH 5.75. The input and output concentrations of xylose and glucose are indicated. The hatched bars represent the volumetric productivity (g ethanol/L/h)

Table 7 Summary of Rec *Zymomonas* Continuous Fermentation Parameters

Strain	pH	Xylose	Glucose	max growth yield	m_e	reference
39676	5.75	4.0	0.8	0.042	1.13	ABAB 70-72, 1998
39676	5.75	4.0	0.8	0.032	0.11	C60/62 Final Rep Fig D-20
"adapted"	5.75	4.0	0.8	0.033	0.12	C107 (ZM)
	5.75	4.0	0.8	0.032	0.42	C109 (CSL) Fig 7 ABAB/99
+ 0.4% Ac	5.75	4.0	0.8	0.023	0.54	C115
	5.75	4.0	1.4	0.031	1.1	C131
	5.75	3.0	1.8	0.030	0.46	C111/112
+ 0.4% Ac	5.75	3.0	1.8	0.029	1.0	C114/116
	5.75	2.5	2.5	0.031	0.93	C122/125
CP4:pZB5	5.75	4.0	4.0	0.02	-0.05	C134
CP4:pZB5 + 0.2% Ac	5.75	4.0	4.0	0.014	0.83	C135
CP4:pZB5	5.0	4.0	4.0	0.079	2.5	P. Rogers 20th Symp

PART 6

The work described in Part 6 is related to Task #5 of the Statement of Work. The objective was to assess the effect of ethanol on chemostat performance using two feed sugar ratios and to assess the effect of ethanol on media containing 0.2% (w/v) acetic acid.

This work has already been described in the following contexts:

(i) Technical Progress Report #5 (Fig. 7)
(ii) Technical Progress Report #6 (Figs. 4 and 5)
(iii) Technical Progress Report #7 (Fig.1)

Effect of ethanol on adapted strain in chemostat culture

Figure 6-1A is a time-course of a continuous fermentation with the adapted strain with a 1% cCSL-medium containing 1.8mM $MgSO_4$ and 4% xylose + 0.8% glucose and 1.2% (w/v) ethanol. The initial dilution rate was 0.04/h, but because the effluent xylose had risen rapidly within the first few days to relatively high levels (20-25g/L) the feed pump was stopped after 6 days - during the 46h period that the pump was off the xylose level decreased somewhat but rose again once the pump was turned back on. At day 10 an additional 0.8% glucose was added to the feed reservoir - thus the medium was 4% xylose and 1.6% glucose. After 2 weeks the xylose concentration levelled off at about 15 g/L (Fig 6-1A). This pattern of relatively high xylose could not be directly attributable to the exogenous ethanol in the medium since similar behaviour had been observed with media lacking any inhibitory substances. The effluent ethanol concentration shown in Fig 6-1A represents the total ethanol (exogenous + endogenous). When the experiment was terminated after 22 days of operation, the ethanol process yield was 0.38 g/g and the metabolic yield was 0.50 g/g.

Fig. 6-1B shows a similar experiment in which the amount of ethanol in the medium was doubled to 2.4% (w/v). The glucose concentration was 0.8% and the dilution rate was kept constant at 0.04/h. The effluent xylose remained relatively high in the range 20-25g/L (45-50% utilization) during the first week of operation, thereafter it declined gradually to a level of about 10g/L. The endogenous ethanol level was 17 g/L. The process ethanol yield was 0.35 g/g and the metabolic yield was 0.45 g/g (Fig 6-1B). The gradual "adaptation" is problematic; however, it was concluded that these levels of ethanol was not inhibitory.

Effect of ethanol + acetic acid on the adapted strain in chemostat culture

Figure 6-2 shows a time course of an experiment designed to study the effect of a combination of 1.2% ethanol plus 0.2% acetic acid. The dilution rate was maintained at 0.04/h Because the effluent xylose had risen rapidly within the first few days to relatively high levels (22g/L) the feed pump was stopped after 6 days - during the 22h period that the pump was off the xylose level decreased somewhat (15 g/L), but rose again to 23g/L once the pump was turned back on (Fig 6-2).

Figure 6-3 is a plot of the steady-state levels of xylose as afunction of the dilution rate and examines the effect of 1.2% ethanol by comparing two media - one with only 0.2% acetic acid and the other which contained both 0.2% acetic acid and 1.2% ethanol. This plot shows that the ethanol causes a more premature rise in the effluent xylose concentration suggesting that the ethanol may exacerbate the inhibitory effect of acetic acid even at the more permissible pH of 5.75 that was used in these experiments.

Summary of continuous fermentation parameters for maximum growth yield and maintenance energy

Plots of the specific rate of sugar utilization versus the specific growth rate (dilution rate) (so called "Pirt" plots) can be used to derive values for the maximum growth yield (inverse of the slope). In these plots the y-axis intercept represents the maintenance energy co-efficient (m_e) or the amount of sugar metabolized to yield energy to support non-growth associated or "maintenance" activities (including wasting energy). Table 7 summarizes the values for both maximum growth yield and maintenance energy coefficient that were derived from the experiments conducted as part of this work. For comparison purposes, we have included other experiments involving different NREL recombinant Zm strains - namely, 39676:pZB4L and CP4:pZB5. In general these data point to the effect of acetic acid on increasing the maintebnance energy coefficient and a lowering of the growth yield. The values reported recently by Peter Rogers with CP4:pZB5 at pH 5 seem anomolous both with respect to growth yield and the maitenance energy values which are considerably higher than those observed under similar conditions in this work except where the pH was controlled at 5.75 (Table 7). Also somewhat anomolous, in terms of the values generally observed in this work, are the values for growth yield and m_e derived from the work performed at NREL with rec Zm 39676:pZB4L and presented at the 19th *Symposium on Biotechnology* (ref ABAB, 70-72, 1998)

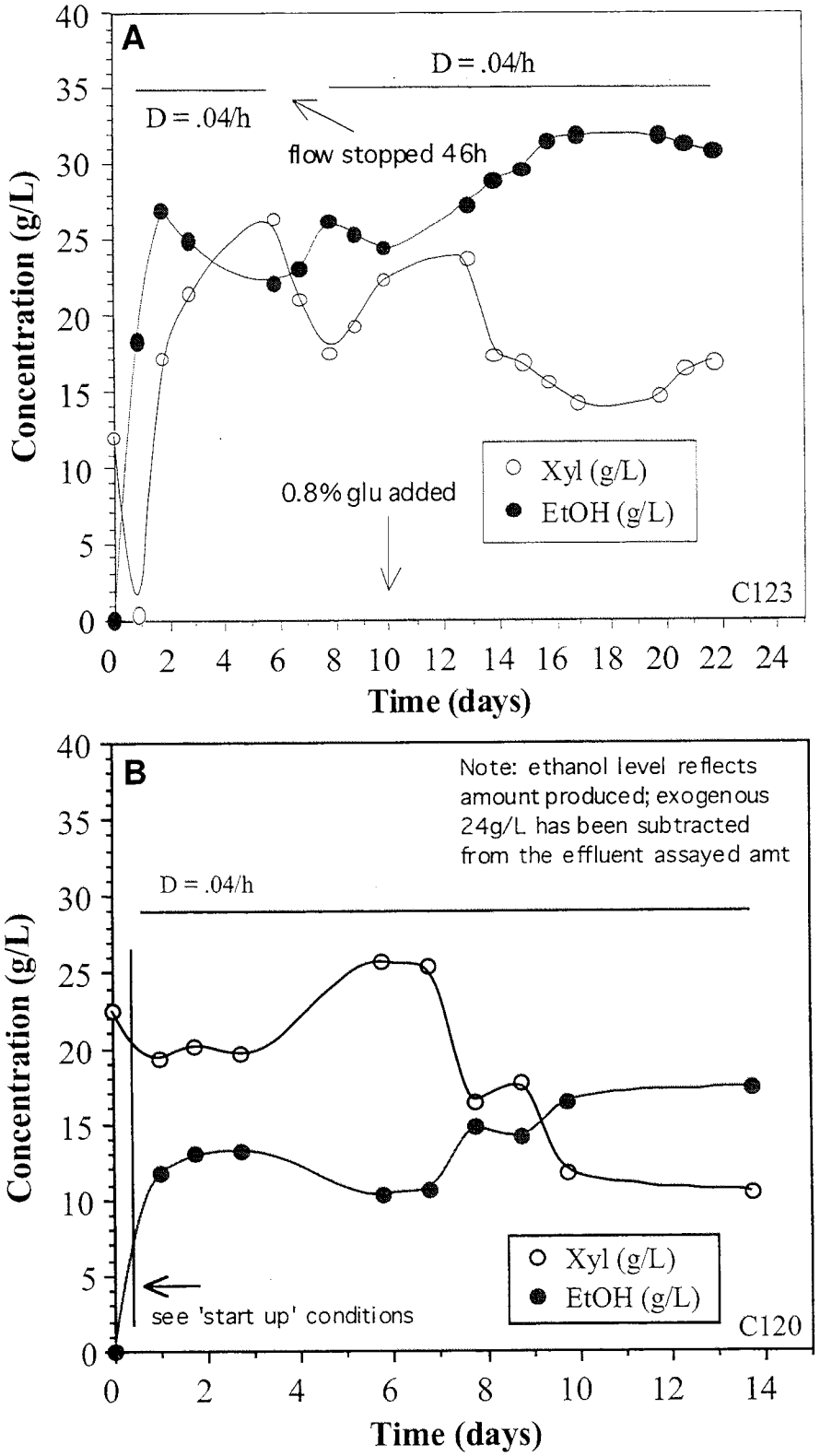


Fig 6-1 Continuous fermentation of "adapted" 39676:pZB4L with 4% xlyose + 0.8% glucose and added ethanol (1% cCSL + 1.8mM Mg) at pH 5.75. (**A**) + 1.2% (w/v) ethanol added to feed, and (**B**) 2.4% (w/v) ethanol added to the feed. Expts C123 and C120

(ref: Fig 7, Prog Report #5; Fig. 4, Prog Report #6)

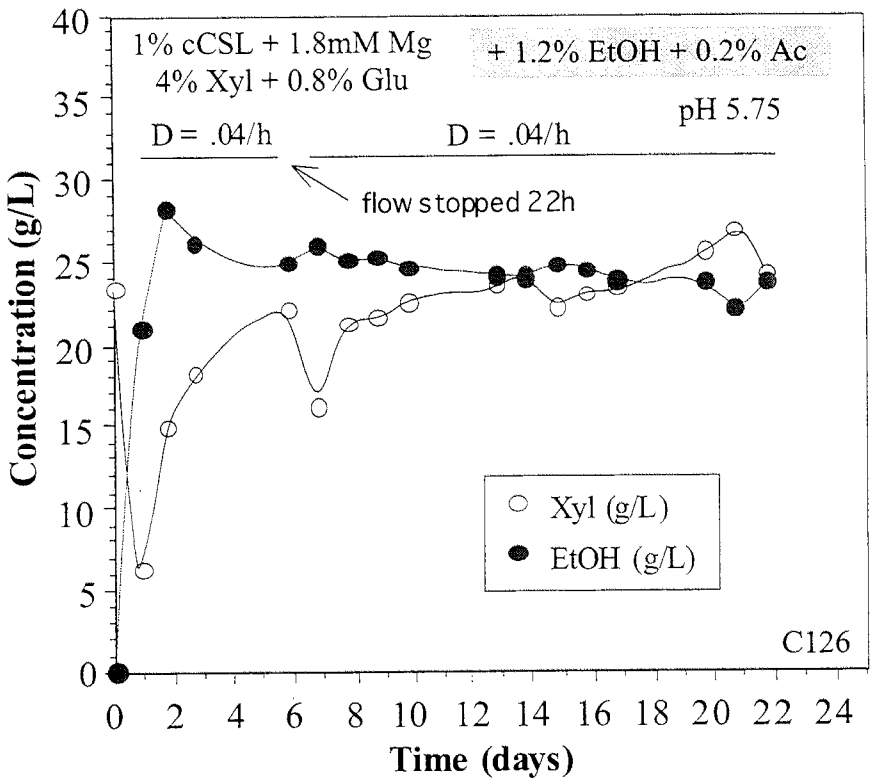


Fig 6-2 Continuous fermentation of "adapted" 39676:pZB4L with 4% xlyose + 0.8% glucose (1% cCSL + 1.8mM Mg) at pH 5.75. The medium reservoir also contained 1.2% (w/v) ethanol and 0.2% (w/v) acetic acid Expt. C126

(ref: Fig 5, Prog Report #6 - Oct 26/98)

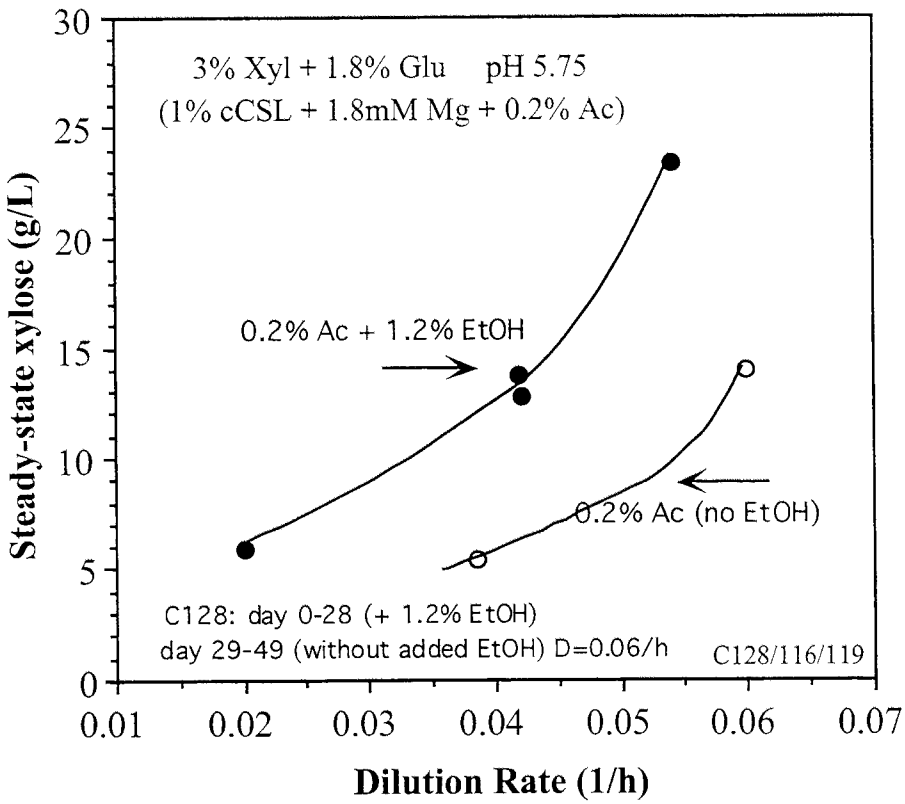


Fig. 6-3 Continuous fermentation of "adapted" 39676:pZB4L with 3% xlyose + 1.8% glucose (1% cCSL + 1.8mM Mg) at pH 5.75. The medium reservoir contained either: (o) 0.2% (w/v) acetic acid, or (o) 0.2% acetic acid + 1.2% ethanol.
Combined steady-state data from Expts. C128/116/119

(ref: Fig 1, Prog Report #7 - Dec. 16/98)

APPENDIX A

Summary of batch fermentations with rZm 39676:pZB4L

OPERATIONAL PARAMETERS FOR BATCH FERMENTATIONS

Sugar conversion by Zymomonas mobilis ATCC 39676:pZB4L

Substrate	SUBSTRATE USE		Products		PRODUCTIVITY				Yield				
Batch #	[Xylose] g/l	[Glucose] g/l	Qsx g X/L/h	Qsg g G/L/h	Biomass g/l	[EtOH] g/l	Xylitol g/l	Acetic g/l	Lactic g/l	Qp g P/L/h	Max. μ	Yp/s gP/gS	Yx/s gX/gS	pH	% C Recov.
Medium Composition: ZM(5g/LYE)(a=fed-batch 5.3% glucose added at 9.5ml/h from 10-48h)															
B103a	34.06	20.22	1.13	0.42	1.92	25.99	2.54	0.00	0.38	0.54	.26	.48	.035	6.0	103
B103b	24.61	20.06	0.51	0.67	2.02	21.58	1.66	0.00	0.44	0.45	.26	.48	.045	6.0	105
Medium Composition: ZM(5g/LYE)(a=fed-batch 5.0% glucose added at 10.0ml/h from 10-48h)															
B104a	60.37	20.66	1.26	0.74	1.98	40.23	1.32	0.00	1.09	0.84	.32	.49	.024	6.0	103
B104b	60.36	19.13	1.26	1.59	1.95	38.58	2.73	0.00	0.89	0.80	.36	.49	.025	6.0	102
Medium Composition: cCSL(a-1%GCP,b-1%NACAN,c-2%NACAN)															
B105a	30.69	8.06	0.64	0.62	1.14	18.80	1.66	0.00	0.49	0.39	.36	.49	.036	6.0	104
B105b	32.61	8.18	0.68	0.58	0.93	19.87	1.81	0.00	0.38	0.41	.36	.49	.030	6.0	103
B105c	35.41	7.81	0.74	0.56	1.23	21.13	2.06	0.00	0.00	0.44	.32	.49	.036	6.0	104
Medium Composition: cCSL(a-1%NACAN,b-1%GPC,c-2%GPC)															
B106a	38.96	7.50	1.08	0.58	0.98	22.48	0.39	0.00	0.28	0.62	.35	.48	.031	6.0	99
B106b	40.92	7.57	1.20	0.63	1.22	23.52	0.26	0.00	0.32	0.69	.27	.49	.031	6.0	99
B106c	40.24	7.26	1.26	0.73	1.31	23.04	0.47	0.00	0.38	0.72	.29	.49	.028	6.0	100
Medium Composition: Zm(5g/L YE + Zymo salts)															
B107a	60.43	8.64	1.12	0.82	1.43	33.12	2.00	0.00	0.89	0.61	.	.48	.031	6.0	100
B107b	55.76	59.74	0.77	2.72	1.67	55.51	2.91	0.00	0.97	0.77	.	.48	.017	6.0	99
B107c	58.09	60.Fruc	0.81	-	1.65	56.68	2.67	0.00	0.95	0.79	.	.48	.016	6.0	-

	Substrate	SUBSTRATE USE		Products		PRODUCTIVITY			Yield					
Batch #	[Xylose] g/l	[Glucose] g/l	Qsx g X/L/h	Qsg g G/L/h	Biomass g/l	[EtOH] g/l	Xylitol g/l	Acetic g/l	Lactic g/l	Qp g P/L/h	Max. μ	Yp/s gP/gS	Yx/s gX/gS	pH	% C Recov.
Medium Composition: cCSL(1%GPC)inocula grown in RM except for B, Initial OD- a=.16,b=.15,c=.27,d=.4															
B108a	40.06	8.30	1.25	0.83	0.95	23.34	0.97	0.00	0.00	0.73	.32	.48	.020	6.0	99
B108b	40.47	8.07	1.26	0.67	0.92	23.22	0.05	0.00	1.66	0.73	.26	.48	.019	6.0	99
B108c	39.99	8.07	1.29	1.35	0.95	23.11	0.00	0.00	1.62	0.75	.30	.48	.020	6.0	100
B108d	40.12	7.95	1.25	1.33	1.00	23.92	0.05	0.00	0.21	0.75	.24	.50	.021	6.0	100
Medium Composition: cCSL+ Zymo salts-(a&b=1%, c=.5%+1.23 g/L$(NH_4)_2HPO_4$,d=1%+1.23g/L$(NH_4)_2HPO_4$)															
B110a	58.54	8.15	1.22	0.91	1.16	31.42	0.99	0.00	2.99	0.65	.33	.47	.025	6.0	100
B110b	57.46	20.28	1.20	1.69	1.40	36.41	1.22	0.00	3.33	0.76	.34	.47	.020	6.0	100
B110c	60.61	20.83	1.78	1.74	1.73	38.58	0.49	0.00	3.01	1.13	.40	.47	.024	6.0	100
B110d	58.89	20.64	1.23	1.72	1.61	37.72	0.89	0.00	2.95	0.79	.32	.47	.024	6.0	100
Medium Composition: 1%cCSL+ Zymo salts															
B111b	35.96	8.51	0.50	0.65	1.01	21.39	0.00	0.40*	1.66	0.30	.34	.48	.025	6.0	100
B111d	29.43	8.59	0.41	0.61	0.84	17.86	0.00	0.77*	2.00	0.25	.32	.47	.027	6.0	100
Medium Composition: ZM (5 g/L YE + .8 g/L NH_4Cl+Zymo salts)															
B112b	0.00	48.37	-	4.04	1.36	23.63	0.13	0.00	0.00	1.58	.35	.49	.028	5.75	99
B112d	38.05	8.24	1.27	1.03	1.38	22.81	1.20	0.00	0.49	0.76	.29	.49	.030	5.75	104
Medium Composition: b=ZM															
B113b	35.11	0.00	0.73	-	0.68	17.08	0.04	0.00	2.28	0.36	.15	.49	.019	5.75	104
Medium Composition: a=1%cCSL,b=.25%cCSL+ 1.23g/L $(NH_4)_2HPO4$,c&d=ZM															
B114c	76.25	24.63	1.03	1.45	2.25	47.31	5.22	0.51	4.32	0.64	.29	.47	.018	5.75	106
B114d	14.63	18.78	0.20	0.25	0.86	15.70	0.00	0.00	0.46	0.21	.09	.47	.027	5.75	9

	Substrate	SUBSTRATE USE		Products				PRODUCTIVITY		Yield				
Batch #	[Xylose] g/l	[Glucose] g/l	Qsx g X/L/h	Qsg g G/L/h	Biomass g/l	[EtOH] g/l	Xylitol g/l	Acetic g/l	Lactic g/l	Qp g P/L/h	Max. μ	Yp/s gP/gS	Yx/s gX/gS	pH	% C Recov.
Medium Composition: b=1%cCSL,d=.25%cCSL+ 1.23g/L $(NH_4)_2HPO_4$)															
B116b	40.45	7.65	1.35	0.96	0.91	23.46	0.05	0.00	1.74	0.78	.27	.49	.021	5.75	101
B116d	40.41	8.01	0.84	0.73	0.69	22.86	0.02	0.00	1.04	0.48	.24	.48	.026	5.75	102
Medium Composition: ZM1(1.50% EtOH added to b)															
B117a	77.52	19.93	1.08	1.53	1.83	46.08	4.86	0.00	2.08	0.64	.29	.47	.026	5.75	102
B117b	63.84	19.75	0.89	1.65	1.31	39.51	4.53	0.00	2.85	0.55	.31	.47	.023	5.75	103
B117c	80.28	15.96	1.12	1.33	1.94	45.72	4.67	0.00	1.42	0.64	.28	.48	.022	5.75	102
B117d	39.86	20.23	1.42	1.69	1.28	29.57	0.00	0.00	0.05	1.06	.29	.49	.022	5.75	105
Medium Composition: a=1%cCSL,c=.25%cCSL+ 1.23g/L $(NH_4)_2HPO_4$)															
B118a	40.19	7.92	1.18	1.32	1.18	23.33	0.08	0.00	1.45	0.69	.30	.48	.028	5.75	101
B118c	36.82	8.11	0.77	1.35	1.05	21.41	0.15	0.00	1.09	0.45	.26	.48	.022	5.75	99
Medium Composition: ZM)															
B120a	40.72	8.00	0.90	1.33	0.88	23.53	0.40	4.0*	0.28	0.52	.28	.48	.025	6.0	98
B120c	40.24	8.11	0.84	1.01	0.70	23.47	0.80	7.3*	0.23	0.49	.24	.48	.021	6.0	99
B120g	40.38	8.33	1.35	-	1.38	23.60	0.00	0.00	0.78	0.79	.-	.48	.035	5.75	100
Medium Composition: g&h=ZM,b,c&d=1%cCSL,a=.25%cCSL + 1.23g/L $(NH_4)_2HPO_4$															
B121a	31.87	8.16	0.44	0.82	0.61	19.33	0.07	4.0*	2.01	0.27	.32	.48	.030	6.0	101
B121c	24.80	8.20	0.34	0.59	0.63	15.83	0.00	10.0*	1.64	0.22	.26	.48	.025	6.0	101
B121g	0.00	47.90	-	4.79	1.90	22.86	0.00	0.00	0.00	2.29	.36	.48	.040	5.75	98

	Substrate	SUBSTRATE USE			Products				PRODUCTIVITY		Yield			
Batch #	[Xylose] g/l	[Glucose] g/l	Qsx g X/L/h	Qsg g G/L/h	Biomass g/l	[EtOH] g/l	Xylitol g/l	Acetic g/l	Lactic g/l	Qp g P/L/h	Max. μ	Yp/s gP/gS	Yx/s gX/gS	pH	% C Recov.
Medium Composition: 1%cCSL + Zymo salts(parent = a,b &c)															
B125a	40.25	8.90	0.57	0.64	0.73	24.04	0.84	4.0*	0.59	0.35	.33	.49	.019	5.00	100
B125b	40.15	8.85	0.72	0.74	0.96	24.92	1.12	4.0*	1.39	0.44	.36	.50	.034	5.50	105
B125c	40.60	8.58	0.81	0.72	1.06	24.22	1.16	4.0*	1.46	0.48	.33	.49	.022	6.00	104
Medium Composition:2.5ml/LcCSL(NREL)+ Zsalts); g=39676:pZB4L															
B140g	39.91	7.82	0.83	0.78	0.90	23.39	0.00	4.0*	0.90	0.49	.27	.49	.026	6.0	99
Medium Composition: ZM; c=39676:pZB4L															
B151c	22.76	90.64	0.47	1.90	2.02	54.68	1.65	0.00	3.97	1.14	.32	.48	.018	6.0	101
Medium Composition: ZM1; b&d=39676:pZB4L															
B152b	2.26	59.90	0.05	1.25	1.28	29.82	0.00	0.00	0.00	0.62	.20	.48	.021	5.0	97
B152d	3.96	88.67	0.08	1.86	1.46	43.56	0.00	0.00	2.16	0.91	.20	.47	.016	5.75	96

APPENDIX B

Summary of batch fermentations with rZ "adapted" 39676pZB4L

OPERATIONAL PARAMETERS FOR BATCH FERMENTATIONS

Sugar conversion by "adapted" Zymomonas mobilis ATCC 39676:pZB4L

Substrate	**SUBSTRATE USE**		**Products**		**PRODUCTIVITY**		**Yield**								
Batch #	[Xylose] g/l	[Glucose] g/l	Qsx g X/L/h	Qsg g G/L/h	Biomass g/l	[EtOH] g/l	Xylitol g/l	Acetic g/l	Lactic g/l	Qp g P/L/h	Max. μ	Yp/s gP/gS	Yx/s gX/gS	pH	% C Recov.
Medium Composition: 1%cCSL+ Zymo salts(a&c=adapted strain)															
B111a	32.13	8.94	0.45	0.64	1.20	20.10	0.00	0.40*	1.52	0.28	.33	.49	.035	6.0	103
B111c	27.97	9.12	0.39	0.70	1.15	17.58	0.00	0.77*	1.96	0.24	.40	.47	.037	6.0	101
Medium Composition: ZM (5 g/L YE + .8 g/L NH_4Cl+Zymo salts)(a&c=adapted strain)															
B112a	0.00	48.42	-	3.23	1.42	24.12	0.01	0.00	0.05	1.61	.32	.50	.029	5.75	101
B112c	40.34	8.05	1.68	0.81	1.38	24.16	0.00	0.00	0.12	1.01	.27	.50	.029	5.75	101
Medium Composition: a=ZM,c=1%GPC cCSL,d=.25%cCSL+ 1.35g/L $(NH_4)_2HPO4$(a,c&d=adapted strain)															
B113a	24.49	0.00	0.51	-	0.56	12.25	0.14	0.00	2.06	0.26	.13	.50	.036	5.75	110
B113c	30.26	0.00	0.63	-	0.40	14.69	0.14	0.00	2.10	0.31	.18	.48	.013	5.75	104
B113d	16.50	0.00	0.34	-	0.19	7.78	0.15	0.00	0.89	0.16	.08	.47	.012	5.75	100
Medium Composition: a=1%cCSL,b=.25%cCSL+ 1.23g/L $(NH_4)_2HPO4$(a&b=adapted strain)															
B114a	40.11	8.12	0.73	0.90	0.89	23.02	0.14	0.00	2.20	0.42	.25	.48	.020	5.75	100
B114b	40.05	7.86	1.14	0.79	0.79	22.78	0.05	0.00	2.57	0.65	.25	.48	.022	5.75	100
Medium Composition: ZM(adapted strain)a=5.1%glu added at 9.7ml/h,b=dH_20 added at 9.8ml/h															
B115a	65.21	19.86	1.32	1.09	1.95	39.87	2.53	0.00	2.85	0.83	.	.47	.023	5.75	101
B115b	74.14	10.66	1.37	1.23	1.06	39.60	2.42	0.39	2.51	0.75	.	.47	.014	5.75	99
B115c	72.01	10.50	1.50	1.08	1.21	39.20	2.94	0.00	2.26	0.82	.	.48	.015	5.75	101
B115d	55.76	20.49	1.16	1.46	1.13	36.07	2.87	0.00	2.43	0.21	.	.47	.013	5.75	101

	Substrate	SUBSTRATE USE		Products		PRODUCTIVITY			Yield					
Batch #	[Xylose] g/l	[Glucose] g/l	Qsx g X/L/h	Qsg g G/L/h	Biomass g/l	[EtOH] g/l	Xylitol g/l	Acetic g/l	Lactic g/l	Qp g P/L/h	Max. μ	Yp/s gP/gS	Yx/s gX/gS	pH	% C Recov.
Medium Composition: a=1%cCSL,c=.25%cCSL+ 1.23g/L $(NH_4)_2HPO_4$)(a&c=adapted strain)															
B116a	40.45	7.97	1.12	1.00	0.89	23.30	0.02	0.00	1.70	0.65	.22	.48	.020	5.75	100
B116c	40.83	7.90	1.02	0.66	0.66	23.50	0.06	0.00	1.41	0.59	.20	.48	.018	5.75	99
Medium Composition: b=1%cCSL,d=.25%cCSL+ 1.23g/L $(NH_4)_2HPO_4$)(b&d=adapted strain)															
B118b	40.08	8.57	1.05	1.00	1.06	23.42	0.06	0.00	1.72	0.62	.35	.48	.026	5.75	100
B118d	40.20	8.52	1.26	1.04	1.01	23.28	0.07	0.00	1.80	0.73	.29	.48	.022	5.75	100
Medium Composition: a,b&c=1%cCSL,d=.25%cCSL+ 1.23g/L $(NH_4)_2HPO_4$)(adapted strain)															
B119a	40.44	8.03	0.94	0.62	0.87	23.03	0.00	4.0*	2.13	0.54	.30	.48	.018	6.0	99
B119b	40.48	7.87	0.84	0.56	0.81	22.86	0.00	7.5*	2.61	0.48	.35	.47	.017	6.0	100
B119c	37.97	8.06	0.53	0.56	0.66	21.96	0.00	9.7*	2.97	0.31	.26	.48	.014	6.0	101
B119d	40.06	7.78	1.05	0.43	0.91	23.45	0.00	4.0*	2.12	0.62	.29	.49	.019	6.0	103
Medium Composition: ZM(b,d&h=adapted strain)															
B120b	40.61	8.26	1.19	1.36	0.87	23.49	0.70	3.7*	0.05	0.69	.24	.48	.019	6.0	98
B120d	40.32	8.28	1.12	1.04	0.79	23.49	0.20	7.4*	0.05	0.65	.21	.48	.022	6.0	97
B120h	40.26	8.73	1.55	-	1.34	23.58	0.00	0.00	0.70	0.90	.-	.48	.032	5.75	99
Medium Composition: h=ZM,b=1%cCSL(b&h=adapted strain)															
B121b	36.50	8.11	0.51	0.58	0.72	21.22	0.00	10.0*	2.18	0.29	.28	.48	.022	6.0	100
B121h	0.00	48.18	-	3.44	1.71	23.10	0.00	0.00	0.00	1.65	.33	.48	.035	5.75	98

	Substrate	SUBSTRATE USE			Products			PRODUCTIVITY		Yield				
Batch #	[Xylose] g/l	[Glucose] g/l	Qsx g X/L/h	Qsg g G/L/h	Biomass g/l	[EtOH] g/l	Xylitol g/l	Acetic g/l	Lactic g/l	Qp g P/L/h	Max. μ	Yp/s gP/gS	Yx/s gX/gS	pH	% C Recov.
Medium Composition: 1%cCSL + Zymo salts(adapt.=d,g &h)															
B125d	40.83	8.51	0.85	0.61	0.88	24.78	0.24	4.0*	0.57	0.51	.35	.50	.018	5.00	102
B125g	40.01	8.57	0.91	0.71	1.04	24.20	0.25	4.0*	0.85	0.55	.32	.50	.035	5.50	102
B125h	40.57	8.90	1.01	0.64	1.18	24.30	0.89	4.0*	1.39	0.61	.35	.49	.024	6.00	104
Medium Composition: 0.25%cCSL+1.23g/L $(NH_4)_2HPO_4$ +(a= Zymo salts,b=tapH_2O,d=.5g/L$MgSO_4$,g=.175%hydrolysed CSL +Zs,h=.1%cCSL+Zs)(adapt)															
B126a	40.42	8.28	1.01	0.69	0.72	23.84	0.25	0.00	0.55	0.60	.31	.49	.020	5.75	99
B126b	40.24	7.85	1.06	0.65	0.87	23.71	0.23	0.00	0.56	0.62	.24	.49	.022	5.75	100
B126c	37.86	7.95	0.79	0.57	0.65	22.43	0.29	0.00	0.54	0.47	.22	.49	.021	5.75	99
B126d	41.36	8.66	1.03	0.72	0.82	24.32	0.43	0.00	0.52	0.61	.24	.49	.022	5.75	99
B126g	40.21	7.91	1.01	0.66	0.77	23.43	0.37	0.00	0.37	0.59	.27	.49	.020	5.75	99
B126h	36.67	8.18	0.75	0.59	0.68	21.71	0.27	0.00	0.27	0.45	.18	.48	.020	5.75	98
Medium Composition:ZM (d,g&h=adapted)															
B132d	9.68	37.95	0.13	0.53	0.58	21.85	0.00	0.00	0.84	0.30	.25	.46	.017	5.0	93
B132g	33.38	65.74	0.46	1.10	1.57	46.14	1.37	0.00	1.27	0.64	.25	.47	.020	5.75	96
B132h	40.50	54.54	0.56	0.76	1.16	44.47	0.58	4.0*	0.83	0.62	.20	.47	.017	5.75	94
Medium Composition:cCSL(NREL)a-d=2.5ml/L +1.2g/L $(NH_4)_2HPO_4$,B=tap H_2O,c=Z salts,d=Mg,g=10ml/LcCSL+tapH_2O),h=ZM(adapted)															
B137a	32.61	8.45	0.68	0.85	0.73	20.73	0.30	4.0*	1.02	0.43	.31	.50	.030	5.75	104
B137b	33.39	8.47	0.70	0.85	0.76	20.92	0.08	4.0*	1.63	0.44	.34	.50	.027	5.75	104
B137c	35.59	8.48	0.74	0.85	0.75	22.21	0.14	4.0*	1.44	0.46	.34	.50	.025	5.75	104
B137d	34.47	8.54	0.72	0.85	0.86	21.58	0.20	4.0*	1.76	0.45	.36	.50	.029	5.75	105
B137g	40.61	8.82	1.02	0.88	1.05	24.87	0.17	4.0*	1.26	0.62	.28	.50	.026	5.75	104
B137h	40.41	8.22	1.01	1.03	1.03	24.14	0.63	4.0*	1.26	0.60	.28	.50	.030	5.75	104

Substrate		SUBSTRATE USE			Products			PRODUCTIVITY		Yield				
Batch #	[Xylose] g/l	[Glucose] g/l	Qsx g X/L/h	Qsg g G/L/h	Biomass g/l	[EtOH] g/l	Xylitol g/l	Acetic g/l	Lactic g/l	Qp g P/L/h	Max. μ	Yp/s gP/gS	Yx/s gX/gS	pH	% C Recov.
Medium Composition:2.5ml/LcCSL(NREL)+ b=tapH_2O,c=Zsalts,d=1.67mM Mg)adapted;															
B140a	39.13	7.76	0.82	0.62	0.76	23.08	0.00	4.0*	0.53	0.48	.27	.49	.024	6.0	99
B140b	39.37	7.87	0.87	0.66	0.80	23.19	0.00	4.0*	0.62	0.52	.27	.49	.024	6.0	99
B140c	40.43	8.00	1.12	0.73	0.84	23.90	0.00	4.0*	0.54	0.65	.27	.49	.019	6.0	100
B140d	39.66	7.85	1.10	0.72	0.86	23.46	0.00	4.0*	0.51	0.65	.27	.49	.021	6.0	100
Medium Composition:ZM, a,b,d,e=adapted															
B142a	25.91	0.00	0.54	-	0.95	12.47	0.00	0.00	0.22	0.26	.17	.48	.037	6.0	99
B142b	39.66	0.00	0.83	-	0.79	19.03	0.00	0.00	0.09	0.40	.24	.48	.033	6.0	96
B142d	27.56	0.00	0.66	-	0.48	13.21	0.00	0.00	0.05	0.31	.12	.48	.038	6.0	96
B142e	22.36	0.00	0.47	-	0.58	10.73	0.00	0.00	0.09	0.22	.16	.48	.026	6.097	Medium

APPENDIX C

Summary of batch fermentations with rZ CP4pZB5

OPERATIONAL PARAMETERS FOR BATCH FERMENTATIONS

Sugar conversion by Zymomonas mobilis CP4:pZB5

Substrate	**SUBSTRATE USE**		**Products**							**PRODUCTIVITY**			**Yield**		
Batch #	[Xylose] g/l	[Glucose] g/l	Qsx g X/L/h	Qsg g G/L/h	Biomass g/l	[EtOH] g/l	Xylitol g/l	Acetic g/l	Lactic g/l	Qp g P/L/h	Max. μ	Yp/s gP/gS	Yx/s gX/gS	pH	% C Recov.
Medium Composition: ZM(5g/L YE + .8g/L NH_4Cl + Zymo salts)CP4:pZB5															
B109a	80.76	8.01	2.24	0.89	1.68	41.74	2.15	0.00	4.57	0.73	.36	.47	.019	6.0	102
B109b	80.32	19.86	2.59	1.89	2.33	47.25	1.32	0.00	3.74	0.73	.42	.47	.024	6.0	100
B109c	80.66	30.85	1.41	1.81	2.25	53.20	0.15	0.00	5.84	0.75	.34	.48	.024	6.0	101
B109d	74.58	39.79	1.04	1.99	2.40	53.79	2.49	0.00	4.38	0.75	.34	.47	.022	6.0	100
Medium Composition: d=1%cCSL (d=CP4:pZB5)															
B121d	40.67	8.65	0.56	0.62	0.74	23.46	0.00	10.0*	2.37	0.33	.32	.48	.018	6.0	100
Medium Composition: ZM (CP4:pZB5)(1.5% EtOH added to D)															
B122a	40.10	7.98	1.29	1.18	1.39	22.96	0.00	0.00	2.35	0.68	.48	.48	.031	5.75	102
B122b	60.42	8.06	1.26	1.01	1.46	33.03	0.00	0.00	2.49	0.69	.46	.48	.027	5.75	101
B122c	68.99	7.86	0.96	0.76	1.48	36.60	1.40	0.00	1.72	0.51	.36	.48	.029	5.75	99
B122d	80.56	20.22	1.37	1.73	1.89	63.16	0.09	0.00	3.55	0.67	.42	.48	.024	5.75	99
B122g	40.61	60.27	1.27	4.31	2.57	48.20	0.20	0.00	2.50	1.50	.41	.48	.042	5.75	99
B122h	40.53	80.25	0.67	2.36	2.37	58.18	0.19	0.00	3.64	0.81	.43	.48	.024	5.75	100
Medium Composition: ZM (CP4:pZB5)(0.77% glucose added to C at 24h)															
B123a	63.33	8.13	1.17	0.63	1.18	33.92	0.89	0.00	2.16	0.63	.37	.47	.036	5.75	99
B123b	62.05	8.10	1.15	0.68	1.35	33.18	1.66	0.00	2.08	0.61	.36	.47	.039	5.75	100
B123c	69.56	15.55	1.29	0.66	1.57	41.19	1.27	0.00	2.30	0.76	.36	.48	.020	5.75	101
B123d	71.24	16.71	1.32	1.30	1.78	42.20	2.58	0.00	2.69	0.78	.37	.48	.035	5.75	102
B123g	66.59	31.01	1.23	2.07	2.03	46.38	2.27	0.00	2.86	0.86	.40	.48	.029	5.75	101
B123h	66.91	40.65	1.24	2.26	2.32	51.13	2.80	0.00	3.11	0.95	.41	.48	.031	5.75	101

	Substrate	SUBSTRATE USE		Products		PRODUCTIVITY			Yield					
Batch #	[Xylose] g/l	[Glucose] g/l	Qsx g X/L/h	Qsg g G/L/h	Biomass g/l	[EtOH] g/l	Xylitol g/l	Acetic g/l	Lactic g/l	Qp g P/L/h	Max. μ	Yp/s gP/gS	Yx/s gX/gS	pH	% C Recov.
Medium Composition: ZM(CP4:pZB5)(2.0% EtOH added to D)															
B124a	47.55	0.00	0.66	-	0.74	22.97	0.11	0.00	2.55	0.32	.26	.48	.026	5.75	102
B124b	60.54	8.21	1.21	0.82	1.47	32.95	0.09	0.00	3.18	0.66	.40	.48	.027	5.75	101
B124c	60.24	20.12	1.26	1.68	1.69	39.71	0.09	0.00	3.25	0.83	.37	.49	.022	5.75	103
B124d	60.35	20.48	0.84	1.46	1.64	59.69	0.12	0.00	3.34	0.55	.32	.49	.022	5.75	102
B124g	60.94	40.52	0.85	2.89	1.85	49.51	0.12	0.00	3.68	0.69	.34	.49	.022	5.75	101
B124h	60.13	59.43	0.84	2.43	2.03	58.58	0.12	0.00	3.68	0.81	.41	.49	.018	5.75	101
Medium Composition: ZM(a=fedbatch -4.85%glucose fed at 2ml/h for 8-72h)CP4:pZB5(1.5%EtOH added to c)															
B127a	80.64	12.71	1.12	0.86	1.66	44.66	1.32	0.00	2.65	0.62	.34	.48	.027	5.75	100
B127b	80.33	19.84	1.39	1.65	1.79	47.65	0.85	0.00	2.95	0.66	.40	.48	.023	5.75	99
B127c	65.99	19.23	0.92	1.37	1.73	56.21	1.46	0.00	2.28	0.57	.28	.48	.023	5.75	100
B127d	78.66	40.05	1.09	3.34	2.03	57.21	2.28	0.00	3.39	0.79	.36	.48	.024	5.75	101
B127g	58.24	65.02	0.81	1.63	1.10	56.10	0.15	0.00	0.42	0.78	.36	.46	.013	5.00	91
B127h	57.29	65.26	0.80	1.40	1.70	56.80	1.25	0.00	2.25	0.79	.39	.46	.019	5.75	95
Medium Composition:1% c CSL + tapH_2O (CP4:pZB5)															
B130a	27.22	8.19	0.54	1.02	0.44	17.45	0.00	4.0*	0.03	0.35	.33	.49	.043	5.0	98
B130b	27.14	10.23	0.54	1.22	0.45	17.89	0.00	3.9*	0.00	0.36	.33	.48	.038	5.0	95
B130c	27.36	12.23	0.55	1.42	0.49	19.01	0.00	3.9*	0.00	0.38	.34	.48	.036	5.0	95
B130d	28.54	16.60	0.57	1.66	0.68	21.78	0.00	4.0*	0.06	0.44	.36	.48	.035	5.0	96
B130g	31.90	19.67	0.64	1.97	0.80	24.82	0.21	4.0*	0.07	0.50	.36	.48	.037	5.0	96
B130h	30.15	40.94	0.60	2.72	0.87	34.89	0.00	4.0*	0.04	0.70	.39	.49	.020	5.0	97
Medium Composition:ZM (a,b&c=CP4:pZB5)															
B132a	57.45	64.81	1.17	3.61	2.44	57.98	1.26	0.00	0.74	1.17	.43	.47	.022	5.0	97
B132b	67.21	66.01	1.40	3.47	2.46	61.87	1.38	0.00	1.44	1.29	.48	.46	.021	5.75	95
B132c	65.11	57.67	0.80	3.62	1.56	57.62	0.61	4.0*	0.06	0.80	.36	.47	.016	5.75	94

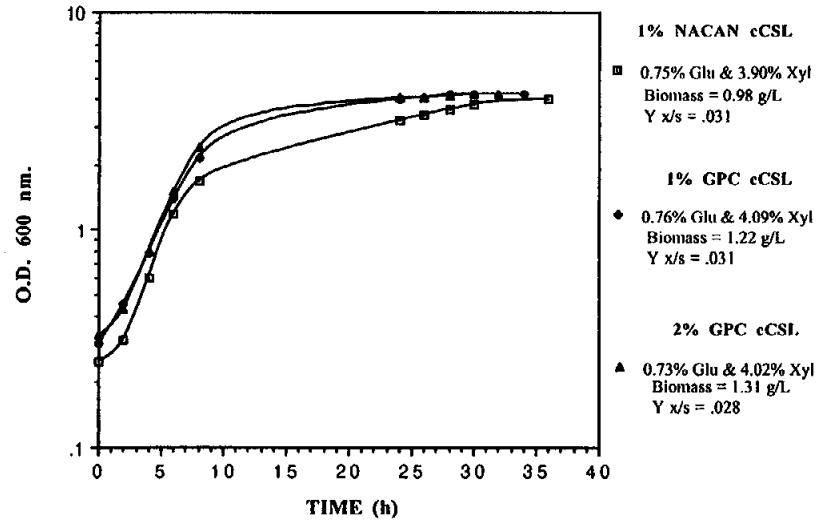
	Substrate	SUBSTRATE USE			Products				PRODUCTIVITY		Yield			
Batch #	[Xylose] g/l	[Glucose] g/l	Qsx g X/L/h	Qsg g G/L/h	Biomass g/l	[EtOH] g/l	Xylitol g/l	Acetic g/l	Lactic g/l	Qp g P/L/h	Max. μ	Yp/s gP/gS	Yx/s gX/gS	pH	% C Recov.
Medium Composition:ZM (CP4:pZB5)															
B134a	52.17	65.45	0.97	3.27	2.45	56.68	0.00	0.00	0.71	1.05	.46	.48	.027	5.75	97
B134b	44.59	65.45	0.83	2.73	2.43	53.36	0.00	0.00	1.88	0.82	.41	.48	.027	5.75	98
B134c	71.58	20.23	1.34	1.90	1.83	44.04	0.00	0.00	0.95	0.99	.45	.48	.028	5.75	97
B134d	64.73	19.79	1.20	1.78	1.88	40.98	0.00	0.00	0.87	0.76	.48	.48	.031	5.75	99
Medium Composition:ZM (CP4:pZB5)															
B135a	43.07	64.04	0.94	2.13	2.28	51.87	0.00	0.00	1.83	1.13	.31	.48	.023	5.75	99
B135b	53.03	65.35	1.30	2.77	2.27	56.92	0.00	0.00	0.88	1.36	.30	.48	.020	5.75	97
B135c	68.37	20.17	1.49	1.55	1.98	42.53	0.00	0.00	0.62	0.92	.29	.48	.023	5.75	97
B135d	62.57	20.97	1.49	1.50	1.81	39.73	0.00	0.00	0.78	0.95	.30	.48	.023	5.75	97
Medium Composition:ZM (CP4:pZB5)															
B136a	42.71	64.89	0.89	2.09	1.89	51.54	0.00	0.00	1.68	1.07	.29	.48	.025	5.75	97
B136b	52.57	64.85	1.11	2.32	2.12	56.63	0.00	0.00	1.51	1.18	.32	.48	.024	5.75	98
B136c	53.58	20.61	1.12	1.21	1.54	35.56	0.00	0.00	1.16	0.74	.29	.48	.043	5.75	97
B136d	65.37	20.38	1.36	1.20	1.82	41.53	0.00	0.00	1.25	0.87	.28	.48	.036	5.75	99
Medium Composition:2.5ml/LcCSL(NREL)+Zsalts (CP4:pZB5)															
B140h	39.35	7.88	1.04	0.76	0.99	23.16	0.00	4.0*	0.87	0.61	.27	.49	.024	6.0	100
Medium Composition:10ml/LcCSL(NREL)+1.67mM Mg (CP4:pZB5)															
B141a	39.70	7.46	0.83	0.73	1.01	23.18	0.00	4.0*	0.65	0.48	.39	.49	.030	6.0	100
B141b	40.56	11.95	1.07	0.96	1.30	26.11	0.00	4.0*	0.70	0.69	.42	.50	.028	6.0	102
B141c	40.57	16.27	1.07	1.12	1.36	28.66	0.00	4.0*	0.86	0.75	.42	.50	.029	6.0	103
B141d	40.49	19.63	0.90	1.35	1.26	29.60	0.00	4.0*	0.94	0.67	.42	.49	.026	6.0	100

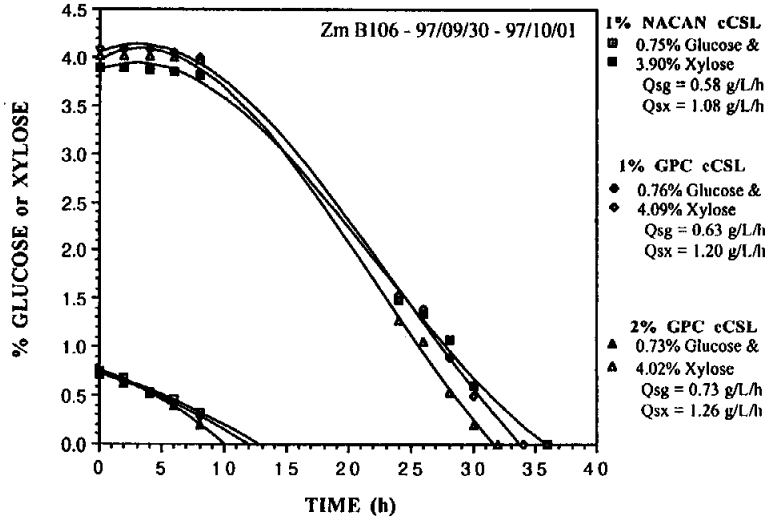
	Substrate	SUBSTRATE USE		Products		PRODUCTIVITY			Yield					
Batch #	[Xylose] g/l	[Glucose] g/l	Qsx g X/L/h	Qsg g G/L/h	Biomass g/l	[EtOH] g/l	Xylitol g/l	Acetic g/l	Lactic g/l	Qp g P/L/h	Max. μ	Yp/s gP/gS	Yx/s gX/gS	pH	% C Recov.
Medium Composition: ZM, CP4:pZB5															
B142c	38.56	0.00	0.88	-	0.74	18.54	0.00	0.00	0.09	0.42	.21	.48	.030	6.0	97
Medium Composition: ZM,CP4:pZB5															
B143a	0.00	11.13	-	1.39	0.67	5.56	0.00	0.00	0.06	0.70	.63	.50	.060	6.0	106
B143b	0.00	16.37	-	1.64	0.86	8.18	0.00	0.00	0.09	0.82	.63	.50	.053	6.0	105
B143c	0.00	20.35	-	2.04	1.08	10.17	0.00	0.00	0.10	1.02	.55	.50	.051	6.0	105
B143d	0.00	41.03	-	3.73	1.53	20.52	0.00	0.00	0.19	1.87	.45	.50	.037	6.0	103
Medium Composition: ZM; CP4:pZB5															
B145a	6.27	0.00	0.52	-	0.25	3.14	0.00	0.00	0.11	0.26	.28	.50	.040	6.0	104
B145b	12.17	0.00	0.72	-	0.39	6.09	0.00	0.00	0.16	0.36	.28	.50	.032	6.0	103
B145c	12.27	0.00	0.68	-	0.38	6.05	0.00	0.00	0.19	0.34	.28	.50	.031	6.0	102
B145d	18.35	0.00	0.48	-	0.58	8.94	0.00	0.00	0.37	0.24	.29	.49	.042	6.0	101
Medium Composition: ZM; c & d = CP4:pZB5															
B146c	10.23	2.84	0.64	0.95	0.54	6.60	0.00	0.00	0.00	0.41	.29	.50	.041	6.0	104
B146d	11.10	1.82	0.65	0.61	0.47	6.49	0.00	0.00	0.08	0.38	.29	.50	.036	6.0	103
Medium Composition: ZM; a, b, c, & d = CP4:pZB5															
B147a	0.00	26.09	-	2.17	1.58?	13.05	0.00	0.00	0.98	1.09	.37	.50	.061	6.0	109
B147b	10.01	0.00	0.59	-	0.41	5.04	0.00	0.00	0.22	0.30	.20	.50	.041	6.0	106
B147c	26.72.	0.00	0.53	-	0.53	12.09	0.00	0.00	0.94	0.25	.19	.48	.024	6.0	100
B147d	41.12	0.00	0.67	-	0.57	15.64	0.00	0.00	1.40	0.33	.20	.49	.024	6.0	101

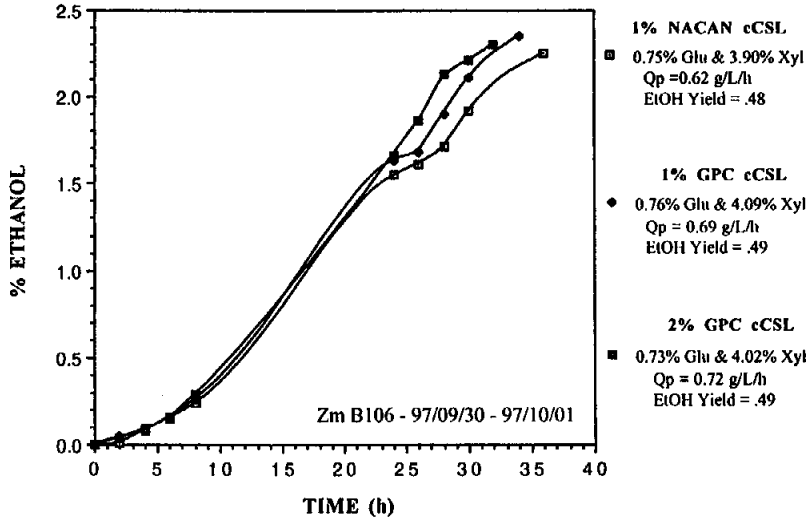
	Substrate	SUBSTRATE USE			Products				PRODUCTIVITY		Yield			
Batch #	[Xylose] g/l	[Glucose] g/l	Qsx g X/L/h	Qsg g G/L/h	Biomass g/l	[EtOH] g/l	Xylitol g/l	Acetic g/l	Lactic g/l	Qp g P/L/h	Max. μ	Yp/s gP/gS	Yx/s gX/gS	pH	% C Recov.
Medium Composition: ZM; a=10ml/h DH_2O; b&e=10ml/h 1.5%Glu; f=10ml/h 5%Glu (CP4:pZB5)															
B149a	34.44	0.00	0.72	-	0.62	13.88	0.00	0.00	0.25	0.29	.24	.49	.035	6.0	90
B149b	28.88	4.15	0.60	0.09	0.90	12.80	0.00	0.00	0.42	0.27	.21	.49	.024	6.0	105
B149e	2.18	2.25	0.36	0.09	0.30	2.17	0.00	0.00	0.03	0.09	.20	.49	.068	6.0	100
B149f	1.68	6.39	0.28	0.27	0.42	3.97	0.00	0.00	0.04	0.17	.22	.49	.052	6.0	99
Medium Composition: a&e=RM; b=ZM-NH_4Cl, c&f=ZM, d=ZM1, f=10ml/h 5%Glu; CP4:pZB5															
B150a	0.00	40.81	-	4.08	1.63	20.69	0.00	0.00	0.00	2.30	.63	.51	.040	6.0	104
B150b	0.00	39.59	-	4.17	1.40	19.77	0.00	0.00	0.00	1.98	.55	.50	.035	6.0	102
B150c	0.00	39.29	-	3.93	1.47	19.98	0.00	0.00	0.00	2.00	.43	.51	.037	6.0	104
B150d	0.00	40.20	-	4.41	1.62	20.29	0.00	0.00	0.00	2.25	.45	.50	.040	6.0	104
B150e	39.14	0.00	0.82	-	0.73	18.81	0.07	0.00	2.07	0.39	.-	.48	.019	6.0	102
B150f	40.71	9.37	1.27	0.29	1.17	22.67	0.03	0.00	0.20	0.71	.-	.49	.025	6.0	92
Medium Composition: b-f=ZM; b,e&f=CP4:pZB5															
B151b	30.66	89.38	0.77	2.78	2.43	57.73	1.65	0.00	4.62	1.44	.43	.48	.022	6.0	102
B151e	0.00	4.39	-	0.09	0.19	2.03	0.07	0.00	0.00	0.04	.19	.46	.019	6.0	97
B151f	0.00	14.23	-	0.30	0.48	6.91	0.20	0.00	0.00	0.14	.30	.49	.025	6.0	100
Medium Composition: ZM1; a,c,e&f=CP4:pZB5															
B152a	22.56	89.82	0.47	3.45	2.38	53.94	0.00	0.00	0.00	1.12	.36	.48	.021	5.0	97
B152c	30.58	89.11	0.80	3.71	2.42	56.50	0.03	0.00	1.25	1.18	.34	.47	.021	5.75	96
B152e	18.26	105.29	0.38	3.51	2.56	59.57	0.00	0.00	0.01	1.24	.36	.48	.022	5.0	97
B152f	31.11	105.59	0.65	4.06	2.71	64.28	0.00	0.00	1.21	1.34	.41	.47	.021	5.75	95

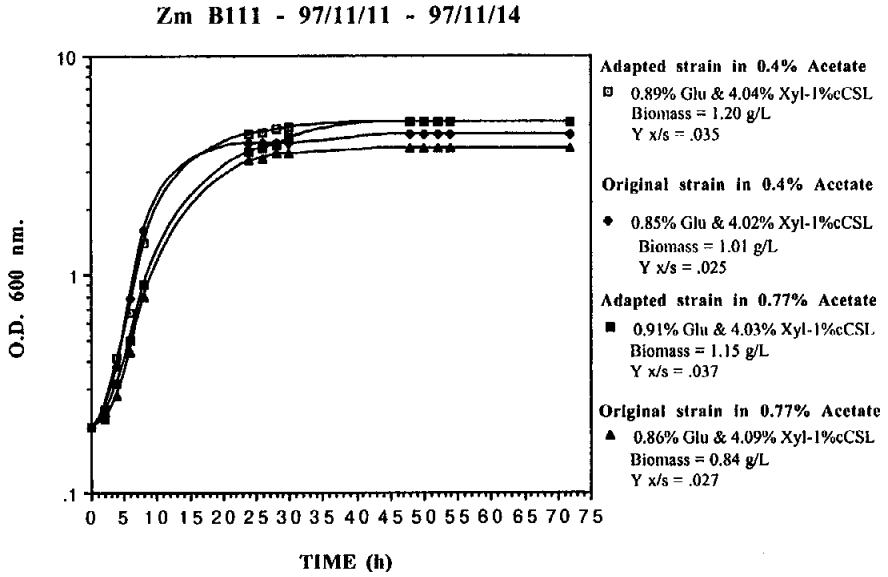
APPENDIX D

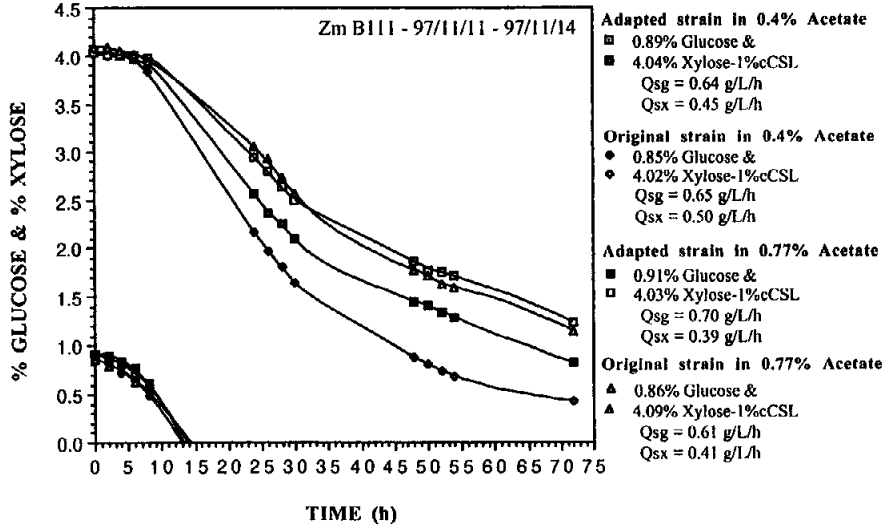
Graphical summaries of batch fermentations for Task 2

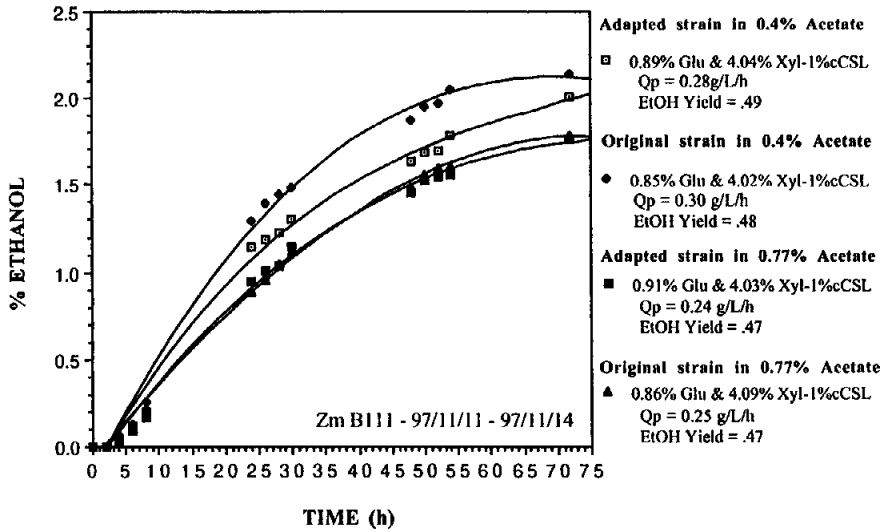

Zm B106 - 97/09/30 - 97/10/01
O.D. 600 nm.
TIME (h)
1% NACAN cCSL
0.75% Glu & 3.90% Xyl
Biomass = 0.98 g/L
Y x/s = .031
1% GPC cCSL
0.76% Glu & 4.09% Xyl
Biomass = 1.22 g/L
Y x/s = .031
2% GPC cCSL
0.73% Glu & 4.02% Xyl
Biomass = 1.31 g/L
Y x/s = .028

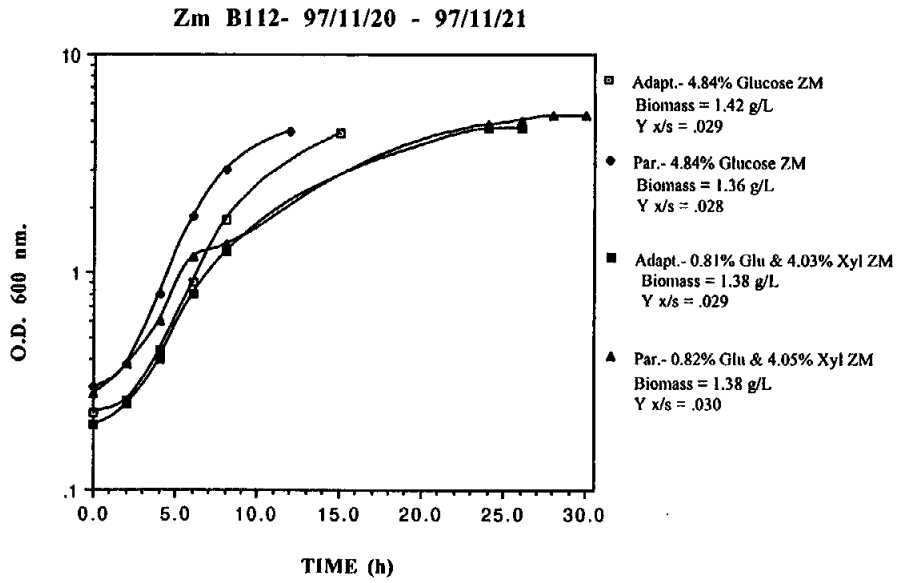

Growth of ATCC 39676:pZB4L in 1% or
2% cCSL + Zymo salts at pH 6.0 & 30°C
Zm B106 - 97/09/30 - 97/10/01
% GLUCOSE or XYLOSE
TIME (h)
1% NACAN cCSL
0.75% Glucose &
3.90% Xylose
Qsg = 0.58 g/L/h
Qsx = 1.08 g/L/h
1% GPC cCSL
0.76% Glucose &
4.09% Xylose
Qsg = 0.63 g/L/h
Qsx = 1.20 g/L/h
2% GPC cCSL
0.73% Glucose &
4.02% Xylose
Qsg = 0.73 g/L/h
Qsx = 1.26 g/L/h

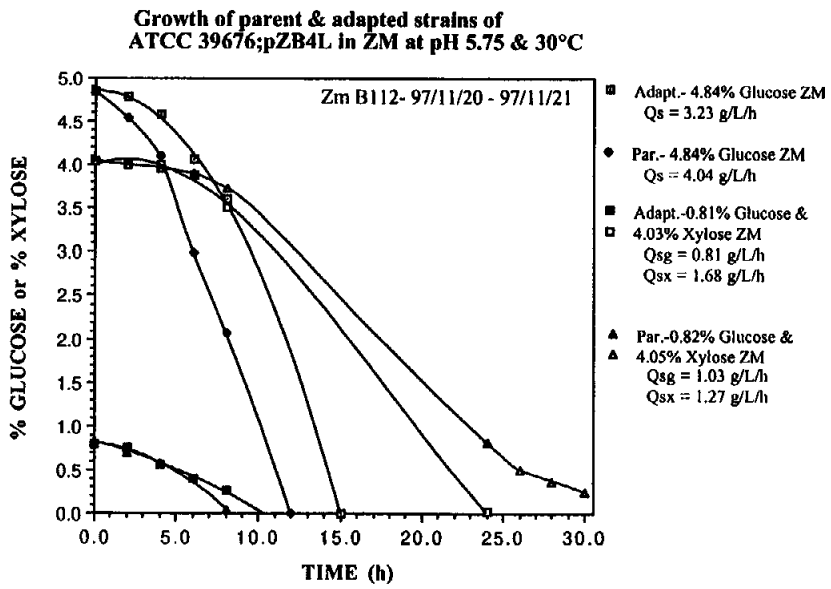

Growth of ATCC 39676:pZB4L in 1% or
2% cCSL + Zymo salts at pH 6.0 & 30°C
% ETHANOL
TIME (h)
Zm B106 - 97/09/30 - 97/10/01
1% NACAN cCSL
0.75% Glu & 3.90% Xyl
Qp =0.62 g/L/h
EtOH Yield = .48
1% GPC cCSL
0.76% Glu & 4.09% Xyl
Qp = 0.69 g/L/h
EtOH Yield = .49
2% GPC cCSL
0.73% Glu & 4.02% Xyl
Qp = 0.72 g/L/h
EtOH Yield = .49

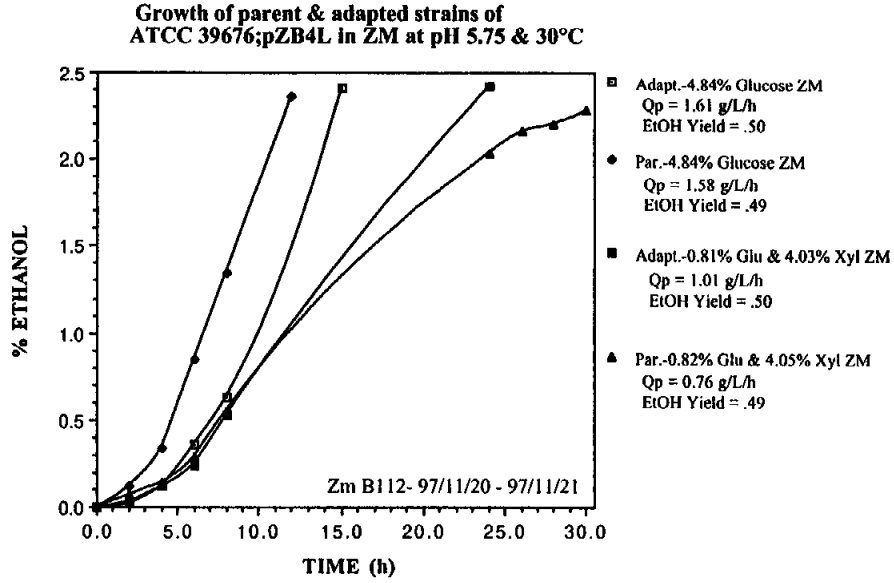

Zm B111 - 97/11/11 - 97/11/14
O.D. 600 nm.
10
1
.1
0 5 10 15 20 25 30 35 40 45 50 55 60 65 70 75
TIME (h)
Adapted strain in 0.4% Acetate
0.89% Glu & 4.04% Xyl-1%cCSL
Biomass = 1.20 g/L
Y x/s = .035
Original strain in 0.4% Acetate
0.85% Glu & 4.02% Xyl-1%cCSL
Biomass = 1.01 g/L
Y x/s = .025
Adapted strain in 0.77% Acetate
0.91% Glu & 4.03% Xyl-1%cCSL
Biomass = 1.15 g/L
Y x/s = .037
Original strain in 0.77% Acetate
0.86% Glu & 4.09% Xyl-1%cCSL
Biomass = 0.84 g/L
Y x/s = .027

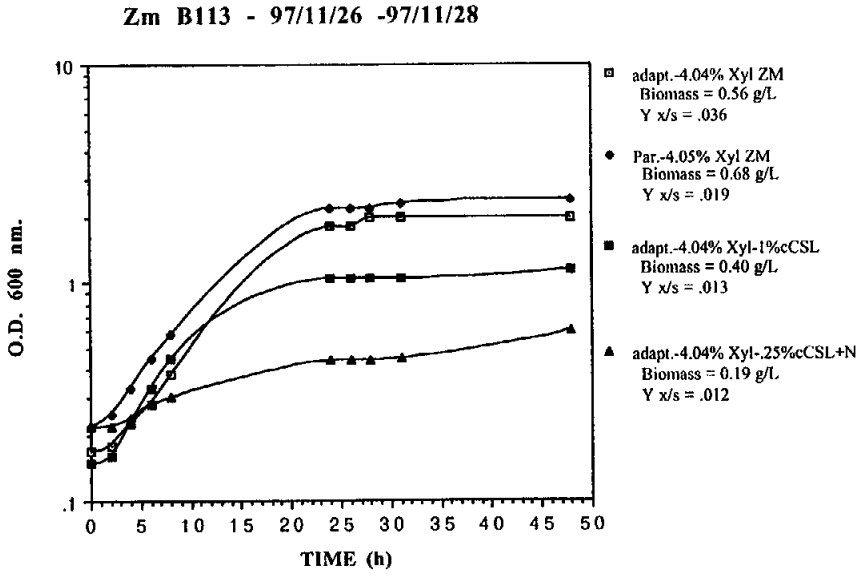

Growth of original & adapted ATCC 39676:pZB4L
in 1% cCSL + Zymo salts + acetate at pH 6.0 & 30°C
Zm B111 - 97/11/11 - 97/11/14
% GLUCOSE & % XYLOSE
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
0 5 10 15 20 25 30 35 40 45 50 55 60 65 70 75
TIME (h)
Adapted strain in 0.4% Acetate
0.89% Glucose &
4.04% Xylose-1%cCSL
Qsg = 0.64 g/L/h
Qsx = 0.45 g/L/h
Original strain in 0.4% Acetate
0.85% Glucose &
4.02% Xylose-1%cCSL
Qsg = 0.65 g/L/h
Qsx = 0.50 g/L/h
Adapted strain in 0.77% Acetate
0.91% Glucose &
4.03% Xylose-1%cCSL
Qsg = 0.70 g/L/h
Qsx = 0.39 g/L/h
Original strain in 0.77% Acetate
0.86% Glucose &
4.09% Xylose-1%cCSL
Qsg = 0.61 g/L/h
Qsx = 0.41 g/L/h

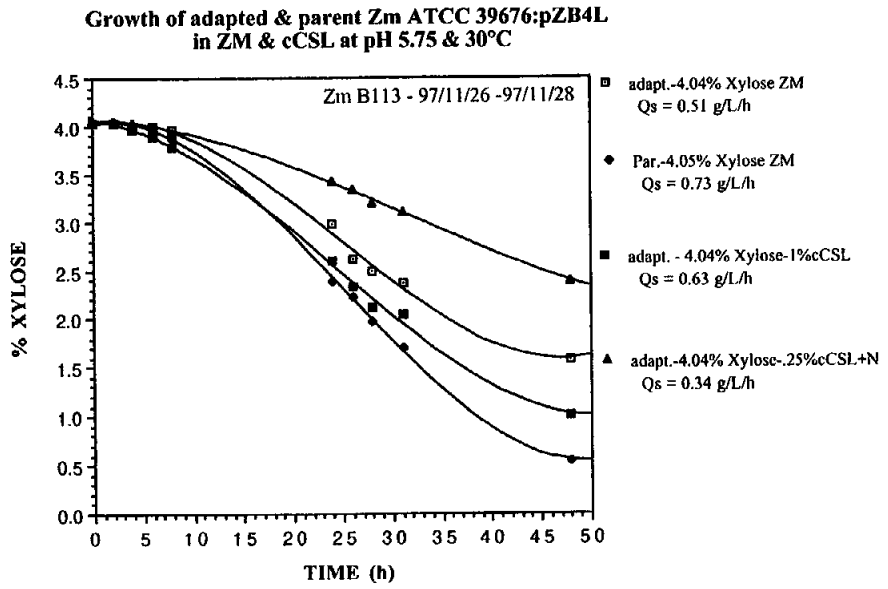

Growth of original & adapted ATCC 39676:pZB4L
in 1% cCSL + Zymo salts + acetate at pH 6.0 & 30°C
% ETHANOL
2.5
2.0
1.5
1.0
0.5
0.0
Zm B111 - 97/11/11 - 97/11/14
0 5 10 15 20 25 30 35 40 45 50 55 60 65 70 75
TIME (h)
Adapted strain in 0.4% Acetate
0.89% Glu & 4.04% Xyl-1%cCSL
Qp = 0.28g/L/h
EtOH Yield = .49
Original strain in 0.4% Acetate
0.85% Glu & 4.02% Xyl-1%cCSL
Qp = 0.30 g/L/h
EtOH Yield = .48
Adapted strain in 0.77% Acetate
0.91% Glu & 4.03% Xyl-1%cCSL
Qp = 0.24 g/L/h
EtOH Yield = .47
Original strain in 0.77% Acetate
0.86% Glu & 4.09% Xyl-1%cCSL
Qp = 0.25 g/L/h
EtOH Yield = .47

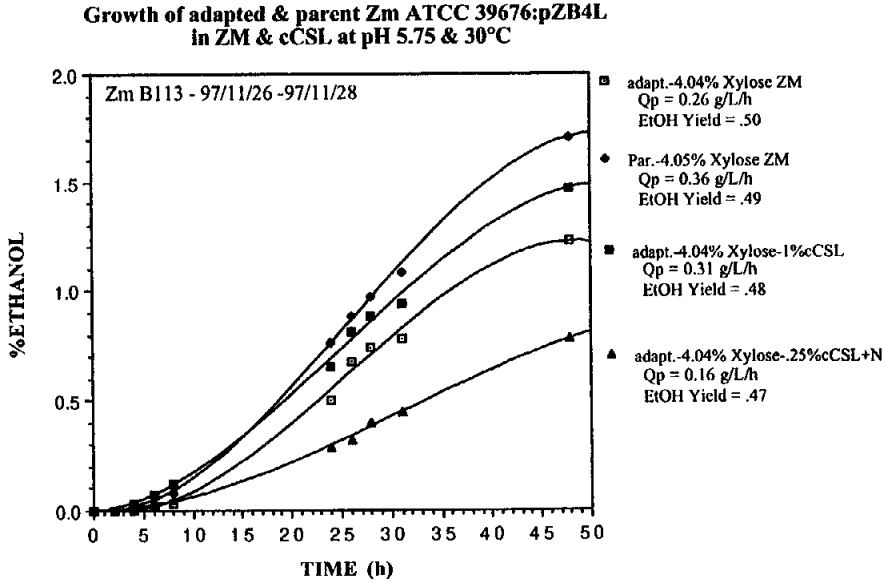

Zm B112- 97/11/20 - 97/11/21
O.D. 600 nm.
TIME (h)
Adapt.- 4.84% Glucose ZM
Biomass = 1.42 g/L
Y x/s = .029
Par.- 4.84% Glucose ZM
Biomass = 1.36 g/L
Y x/s = .028
Adapt.- 0.81% Glu & 4.03% Xyl ZM
Biomass = 1.38 g/L
Y x/s = .029
Par.- 0.82% Glu & 4.05% Xyl ZM
Biomass = 1.38 g/L
Y x/s = .030

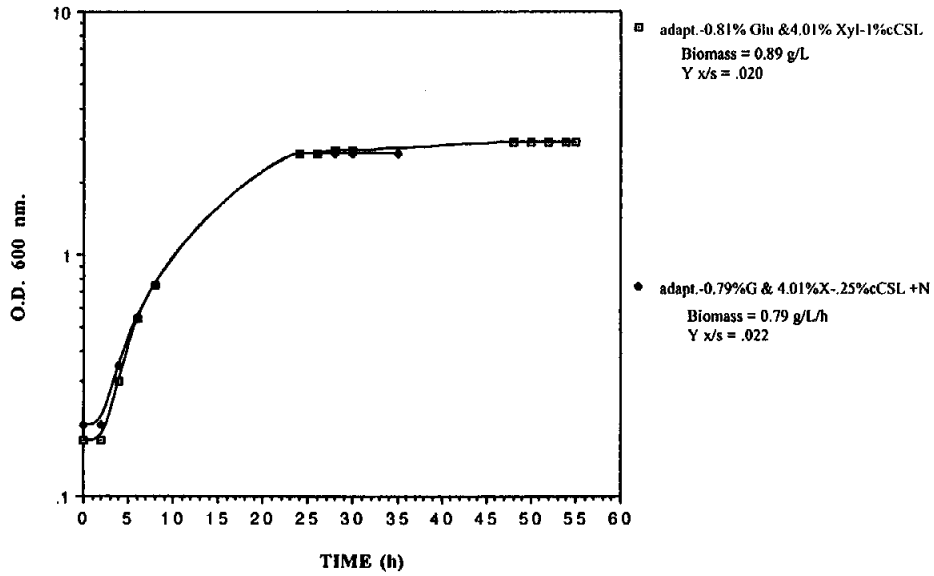

Growth of parent & adapted strains of
ATCC 39676;pZB4L in ZM at pH 5.75 & 30°C
Zm B112- 97/11/20 - 97/11/21
% GLUCOSE or % XYLOSE
TIME (h)
Adapt.- 4.84% Glucose ZM
Qs = 3.23 g/L/h
Par.- 4.84% Glucose ZM
Qs = 4.04 g/L/h
Adapt.-0.81% Glucose &
4.03% Xylose ZM
Qsg = 0.81 g/L/h
Qsx = 1.68 g/L/h
Par.-0.82% Glucose &
4.05% Xylose ZM
Qsg = 1.03 g/L/h
Qsx = 1.27 g/L/h

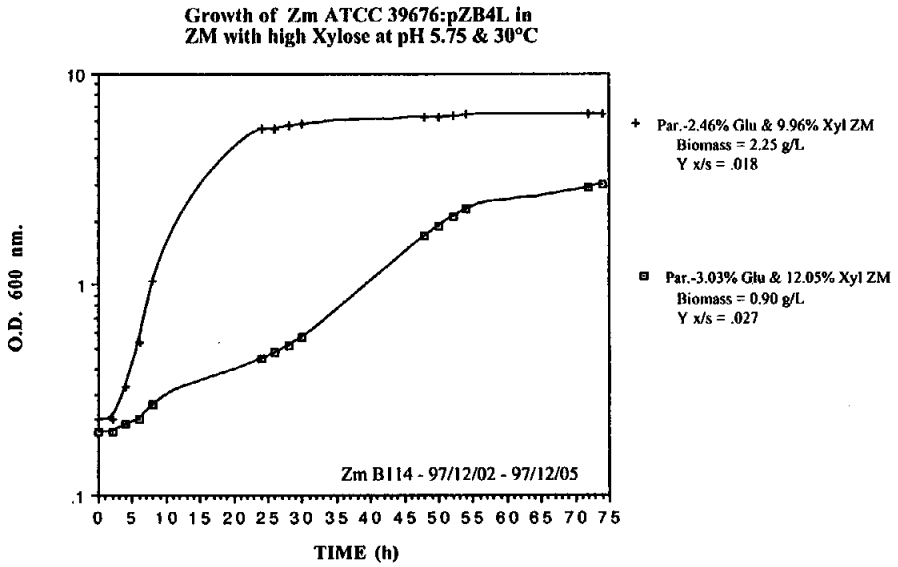

Growth of parent & adapted strains of
ATCC 39676;pZB4L in ZM at pH 5.75 & 30°C
% ETHANOL
TIME (h)
Zm B112- 97/11/20 - 97/11/21
Adapt.-4.84% Glucose ZM
Qp = 1.61 g/L/h
EtOH Yield = .50
Par.-4.84% Glucose ZM
Qp = 1.58 g/L/h
EtOH Yield = .49
Adapt.-0.81% Glu & 4.03% Xyl ZM
Qp = 1.01 g/L/h
EtOH Yield = .50
Par.-0.82% Glu & 4.05% Xyl ZM
Qp = 0.76 g/L/h
EtOH Yield = .49

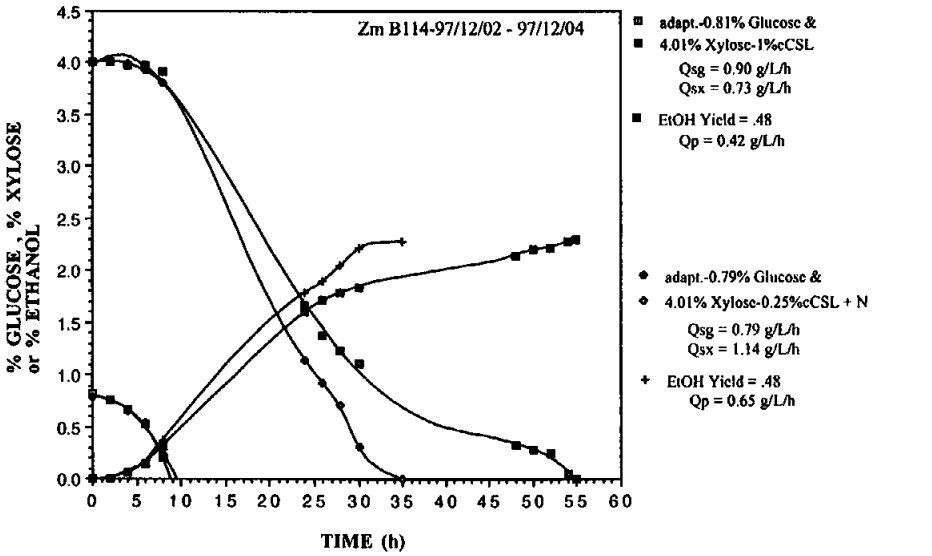

Zm B113 - 97/11/26 -97/11/28
O.D. 600 nm.
TIME (h)
adapt.-4.04% Xyl ZM
Biomass = 0.56 g/L
Y x/s = .036
Par.-4.05% Xyl ZM
Biomass = 0.68 g/L
Y x/s = .019
adapt.-4.04% Xyl-1%cCSL
Biomass = 0.40 g/L
Y x/s = .013
adapt.-4.04% Xyl-.25%cCSL+N
Biomass = 0.19 g/L
Y x/s = .012

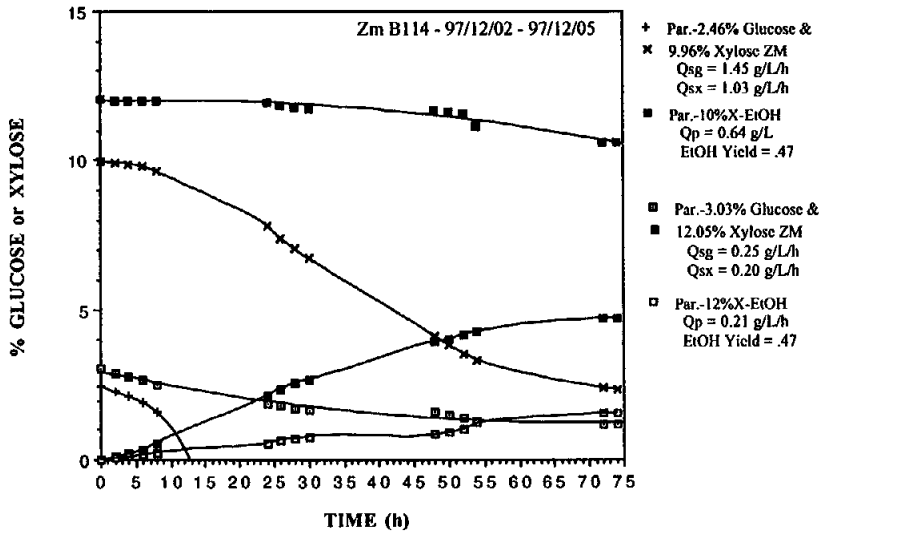

Growth of adapted & parent Zm ATCC 39676:pZB4L
in ZM & cCSL at pH 5.75 & 30°C
Zm B113 - 97/11/26 -97/11/28
% XYLOSE
TIME (h)
adapt.-4.04% Xylose ZM
Qs = 0.51 g/L/h
Par.-4.05% Xylose ZM
Qs = 0.73 g/L/h
adapt. - 4.04% Xylose-1%cCSL
Qs = 0.63 g/L/h
adapt.-4.04% Xylose-.25%cCSL+N
Qs = 0.34 g/L/h

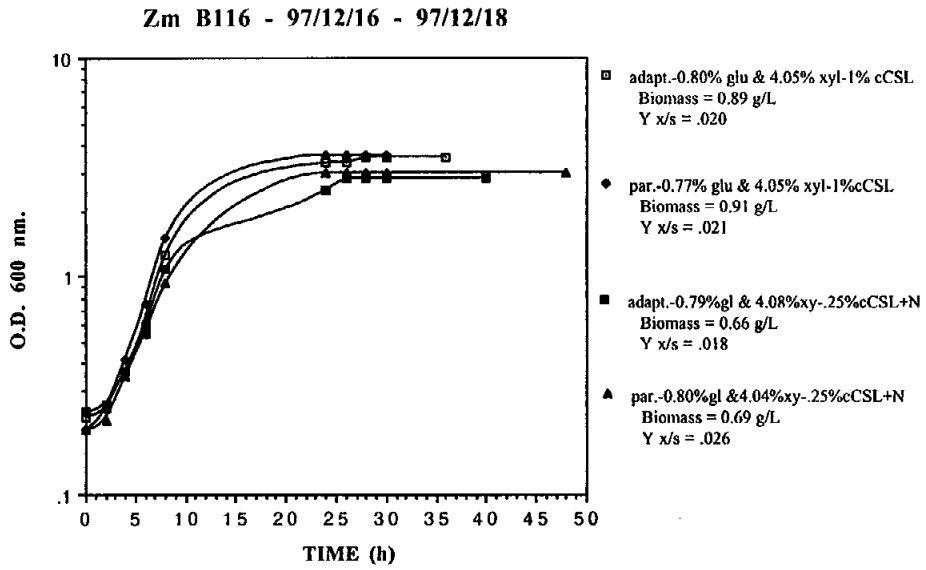

Growth of adapted & parent Zm ATCC 39676:pZB4L
in ZM & cCSL at pH 5.75 & 30°C
Zm B113 - 97/11/26 -97/11/28
%ETHANOL
TIME (h)
adapt.-4.04% Xylose ZM
Qp = 0.26 g/L/h
EtOH Yield = .50
Par.-4.05% Xylose ZM
Qp = 0.36 g/L/h
EtOH Yield = .49
adapt.-4.04% Xylose-1%cCSL
Qp = 0.31 g/L/h
EtOH Yield = .48
adapt.-4.04% Xylose-.25%cCSL+N
Qp = 0.16 g/L/h
EtOH Yield = .47

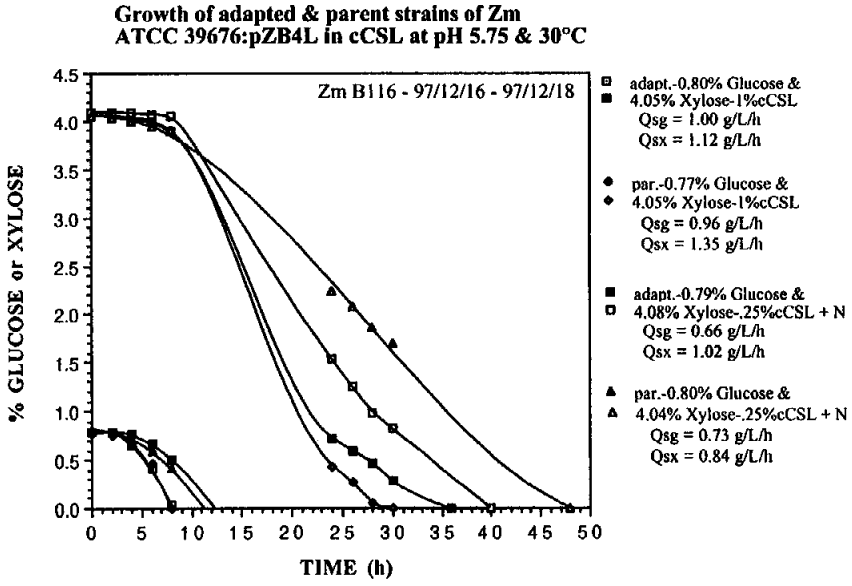

O.D. 600 nm.
TIME (h)
adapt.-0.81% Glu &4.01% Xyl-1%cCSL
Biomass = 0.89 g/L
Y x/s = .020
adapt.-0.79%G & 4.01%X-.25%cCSL +N
Biomass = 0.79 g/L/h
Y x/s = .022

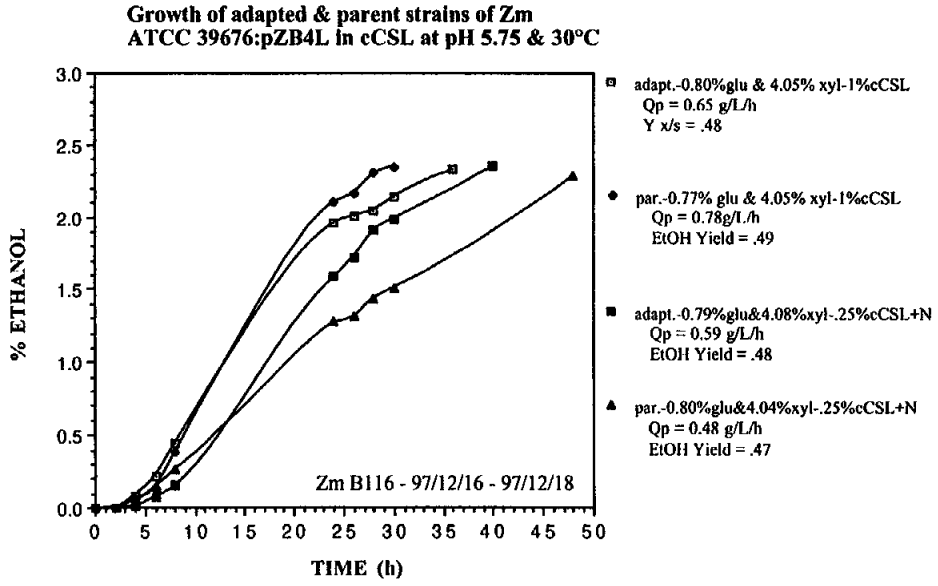

Growth of Zm ATCC 39676:pZB4L in
ZM with high Xylose at pH 5.75 & 30°C
O.D. 600 nm.
TIME (h)
Zm B114 - 97/12/02 - 97/12/05
Par.-2.46% Glu & 9.96% Xyl ZM
Biomass = 2.25 g/L
Y x/s = .018
Par.-3.03% Glu & 12.05% Xyl ZM
Biomass = 0.90 g/L
Y x/s = .027


Growth of Zm ATCC 39676:pZB4L
in cCSL at pH 5.75 & 30°C
% GLUCOSE , % XYLOSE
or % ETHANOL
TIME (h)
Zm B114-97/12/02 - 97/12/04
adapt.-0.81% Glucose &
4.01% Xylose-1%cCSL
Qsg = 0.90 g/L/h
Qsx = 0.73 g/L/h
EtOH Yield = .48
Qp = 0.42 g/L/h
adapt.-0.79% Glucose &
4.01% Xylose-0.25%cCSL + N
Qsg = 0.79 g/L/h
Qsx = 1.14 g/L/h
EtOH Yield = .48
Qp = 0.65 g/L/h

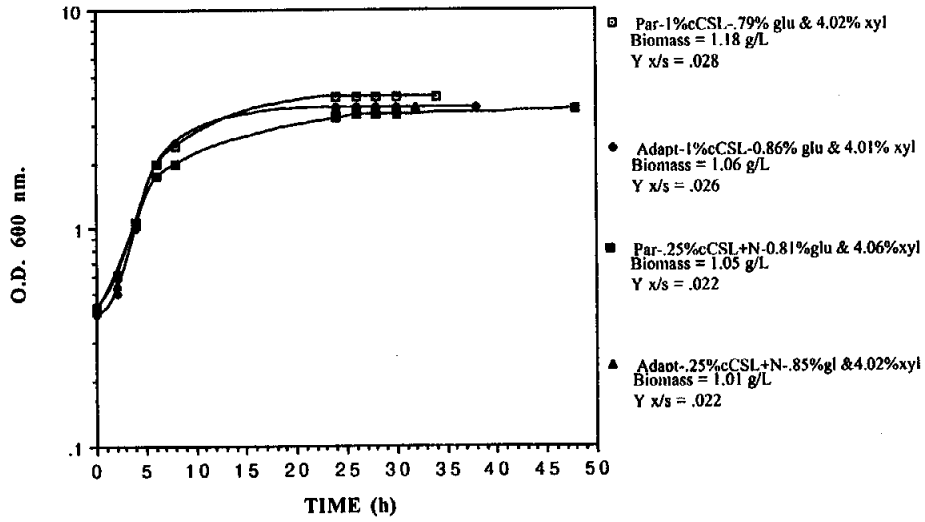

Growth of Zm ATCC 39676:pZB4L in
ZM with high Xylose at pH 5.75 & 30°C
% GLUCOSE or XYLOSE
TIME (h)
Zm B114 - 97/12/02 - 97/12/05
Par.-2.46% Glucose &
9.96% Xylose ZM
Qsg = 1.45 g/L/h
Qsx = 1.03 g/L/h
Par.-10%X-EtOH
Qp = 0.64 g/L
EtOH Yield = .47
Par.-3.03% Glucose &
12.05% Xylose ZM
Qsg = 0.25 g/L/h
Qsx = 0.20 g/L/h
Par.-12%X-EtOH
Qp = 0.21 g/L/h
EtOH Yield = .47


Zm B116 - 97/12/16 - 97/12/18
O.D. 600 nm.
TIME (h)
adapt.-0.80% glu & 4.05% xyl-1% cCSL
Biomass = 0.89 g/L
Y x/s = .020
par.-0.77% glu & 4.05% xyl-1%cCSL
Biomass = 0.91 g/L
Y x/s = .021
adapt.-0.79%gl & 4.08%xy-.25%cCSL+N
Biomass = 0.66 g/L
Y x/s = .018
par.-0.80%gl &4.04%xy-.25%cCSL+N
Biomass = 0.69 g/L
Y x/s = .026

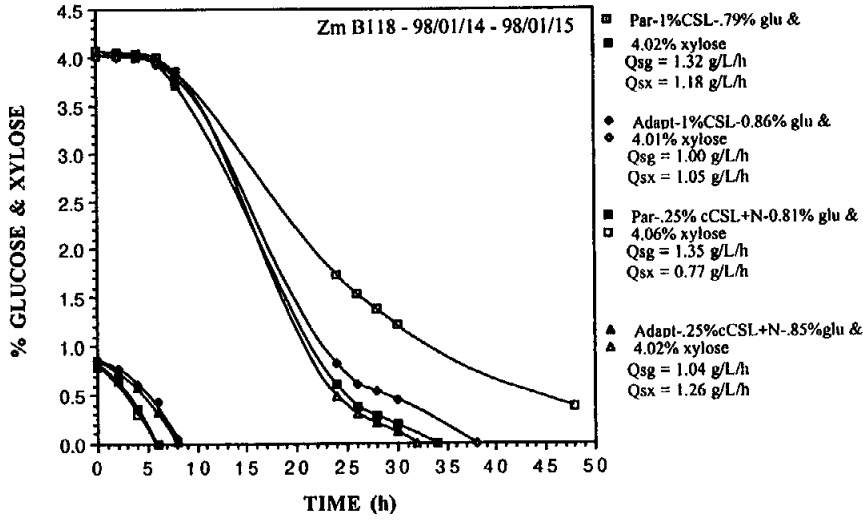

Growth of adapted & parent strains of Zm
ATCC 39676:pZB4L in cCSL at pH 5.75 & 30°C
Zm B116 - 97/12/16 - 97/12/18
% GLUCOSE or XYLOSE
TIME (h)
adapt.-0.80% Glucose &
4.05% Xylose-1%cCSL
Qsg = 1.00 g/L/h
Qsx = 1.12 g/L/h
par.-0.77% Glucose &
4.05% Xylose-1%cCSL
Qsg = 0.96 g/L/h
Qsx = 1.35 g/L/h
adapt.-0.79% Glucose &
4.08% Xylose-.25%cCSL + N
Qsg = 0.66 g/L/h
Qsx = 1.02 g/L/h
par.-0.80% Glucose &
4.04% Xylose-.25%cCSL + N
Qsg = 0.73 g/L/h
Qsx = 0.84 g/L/h


Growth of adapted & parent strains of Zm
ATCC 39676:pZB4L in cCSL at pH 5.75 & 30°C
Zm B116 - 97/12/16 - 97/12/18
% ETHANOL
TIME (h)
adapt.-0.80%glu & 4.05% xyl-1%cCSL
Qp = 0.65 g/L/h
Y x/s = .48
par.-0.77% glu & 4.05% xyl-1%cCSL
Qp = 0.78g/L/h
EtOH Yield = .49
adapt.-0.79%glu&4.08%xyl-.25%cCSL+N
Qp = 0.59 g/L/h
EtOH Yield = .48
par.-0.80%glu&4.04%xyl-.25%cCSL+N
Qp = 0.48 g/L/h
EtOH Yield = .47

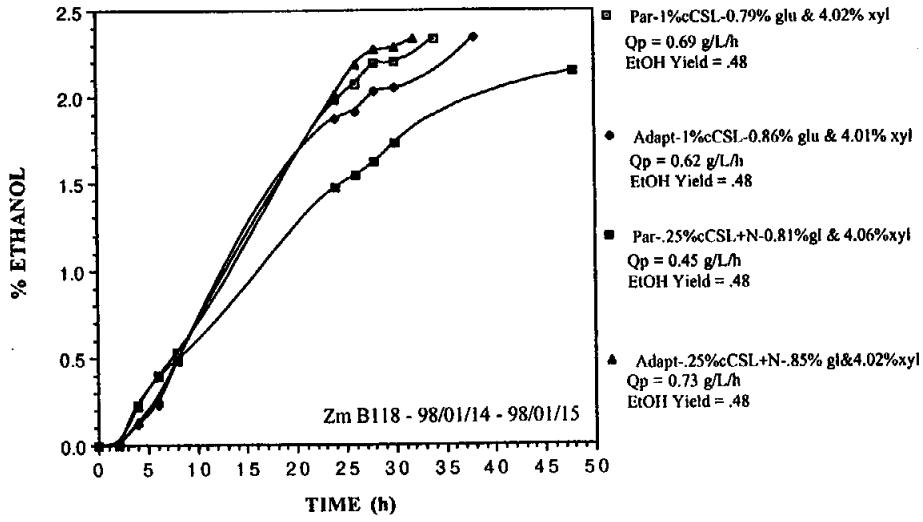
Zm B118 - 98/01/14 - 98/01/15

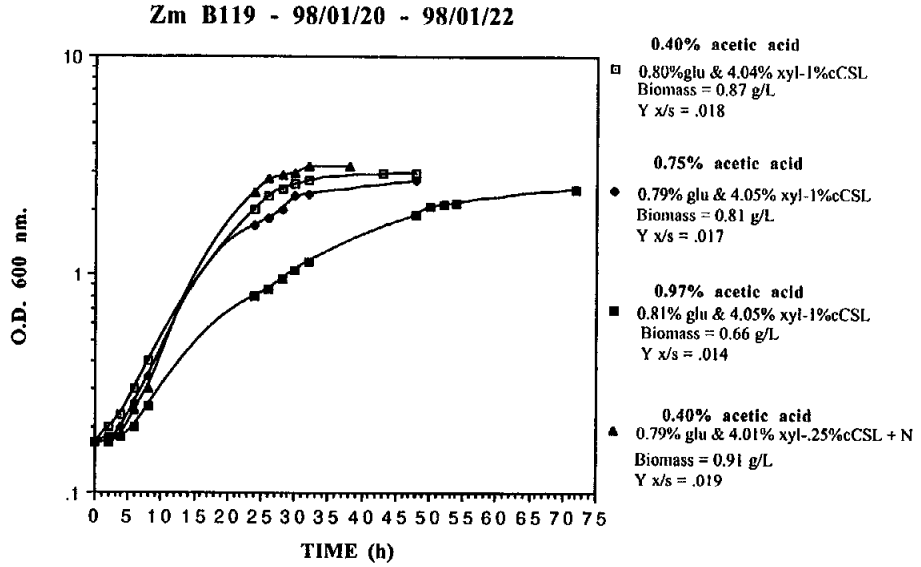


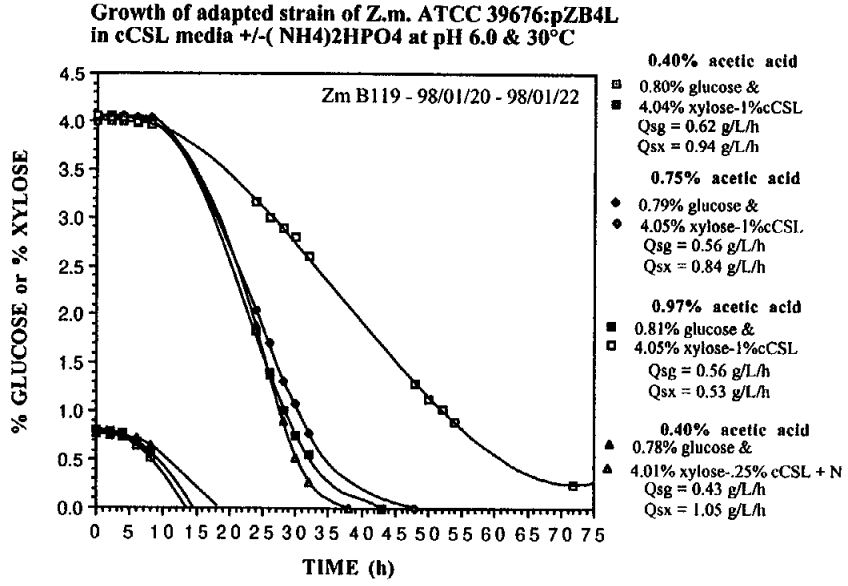
Growth of adapted & parent strains of Z.mobilis ATCC 39676:pZB4L in cCSL Media at pH 5.75 & 30°C

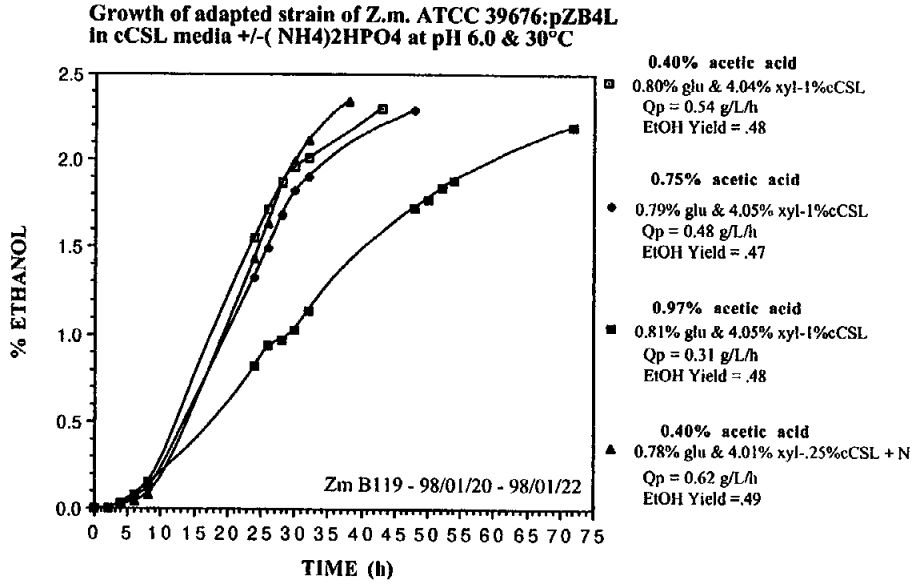


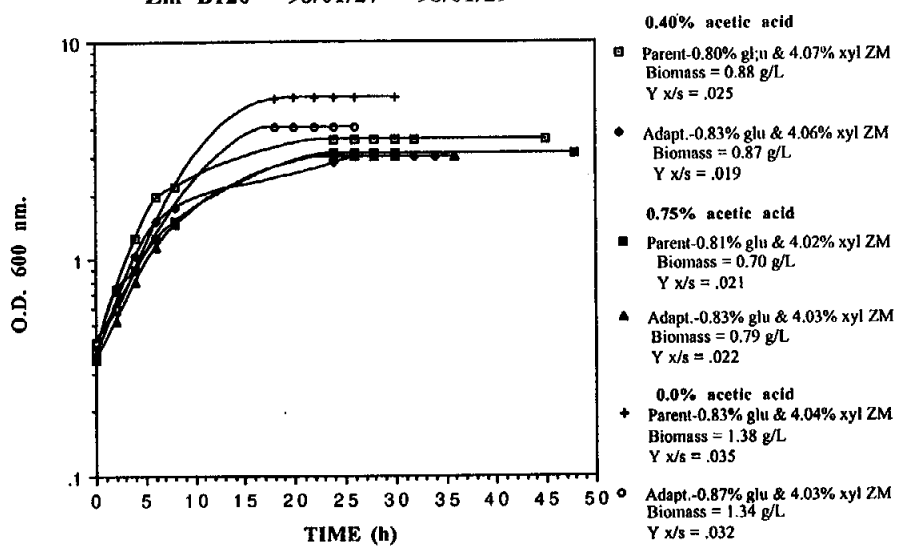
Growth of adapted & parent strains of Z.mobilis ATCC 39676:pZB4L in cCSL Media at pH 5.75 & 30°C

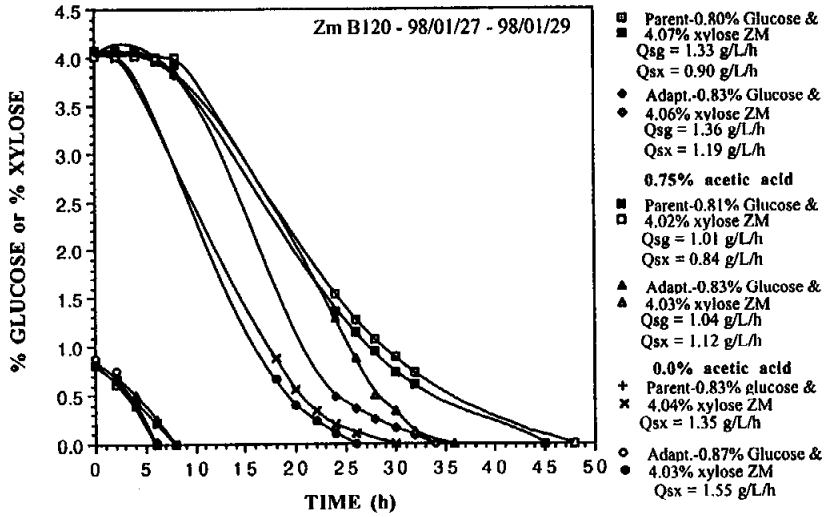


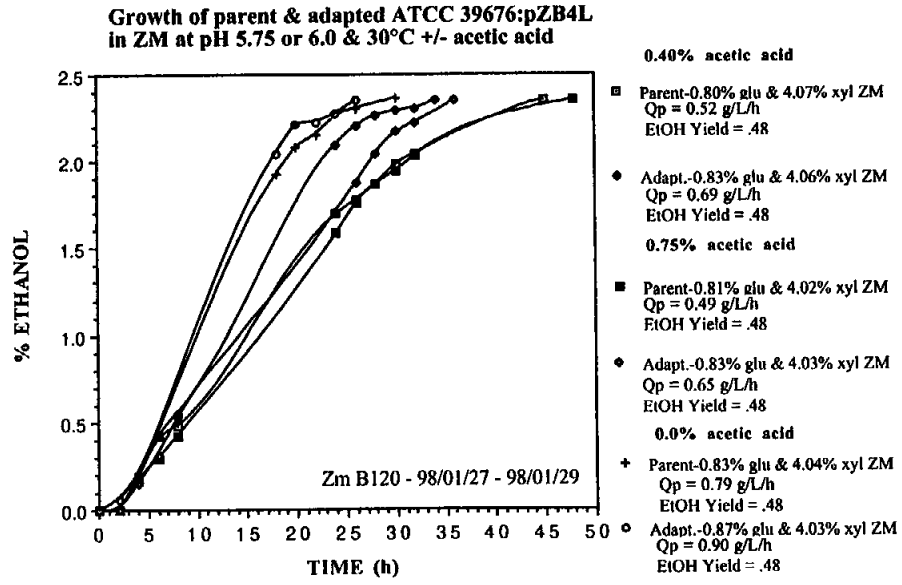

Zm B119 - 98/01/20 - 98/01/22
O.D. 600 nm.
TIME (h)
0.40% acetic acid
0.80%glu & 4.04% xyl-1%cCSL
Biomass = 0.87 g/L
Y x/s = .018
0.75% acetic acid
0.79% glu & 4.05% xyl-1%cCSL
Biomass = 0.81 g/L
Y x/s = .017
0.97% acetic acid
0.81% glu & 4.05% xyl-1%cCSL
Biomass = 0.66 g/L
Y x/s = .014
0.40% acetic acid
0.79% glu & 4.01% xyl-.25%cCSL + N
Biomass = 0.91 g/L
Y x/s = .019

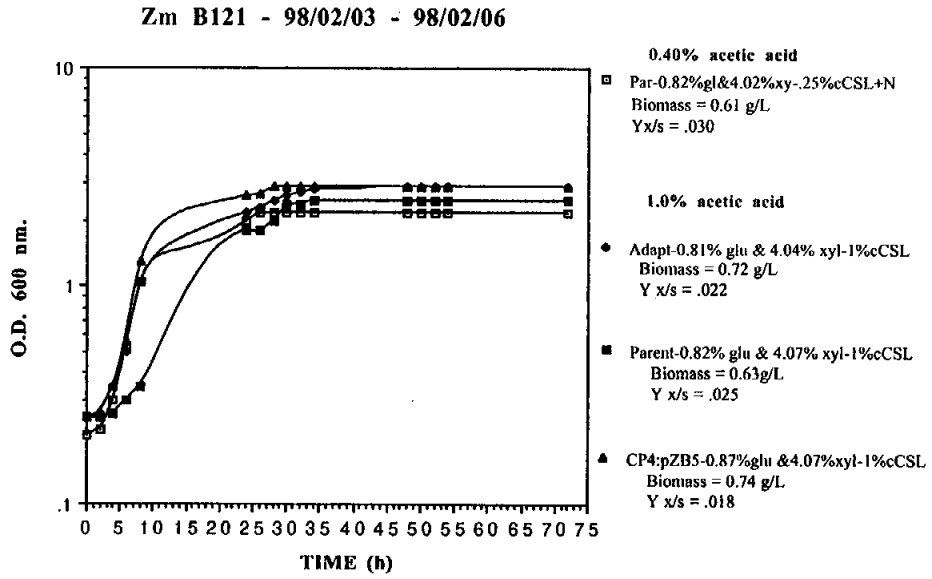

Growth of adapted strain of Z.m. ATCC 39676:pZB4L
in cCSL media +/-(NH4)2HPO4 at pH 6.0 & 30°C
Zm B119 - 98/01/20 - 98/01/22
% GLUCOSE or % XYLOSE
TIME (h)
0.40% acetic acid
0.80% glucose &
4.04% xylose-1%cCSL
Qsg = 0.62 g/L/h
Qsx = 0.94 g/L/h
0.75% acetic acid
0.79% glucose &
4.05% xylose-1%cCSL
Qsg = 0.56 g/L/h
Qsx = 0.84 g/L/h
0.97% acetic acid
0.81% glucose &
4.05% xylose-1%cCSL
Qsg = 0.56 g/L/h
Qsx = 0.53 g/L/h
0.40% acetic acid
0.78% glucose &
4.01% xylose-.25% cCSL + N
Qsg = 0.43 g/L/h
Qsx = 1.05 g/L/h

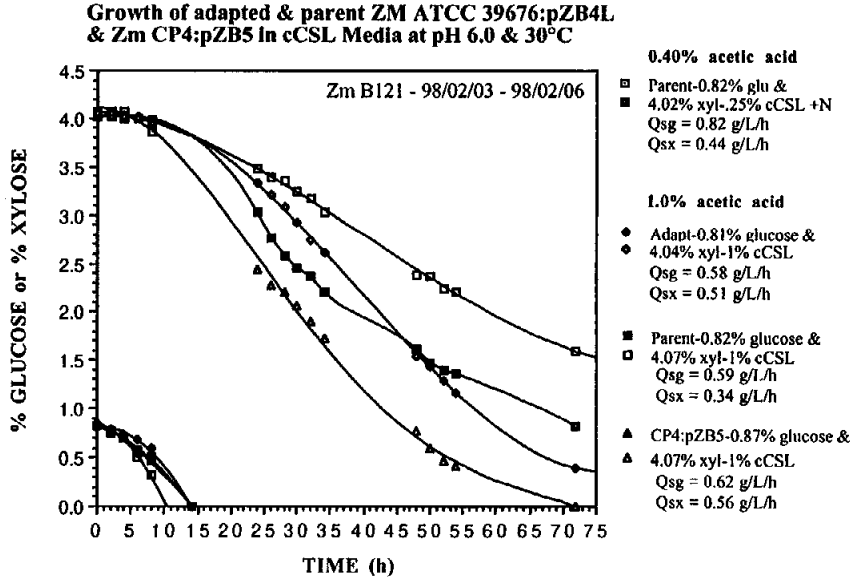

Growth of adapted strain of Z.m. ATCC 39676:pZB4L
in cCSL media +/-(NH4)2HPO4 at pH 6.0 & 30°C
% ETHANOL
TIME (h)
Zm B119 - 98/01/20 - 98/01/22
0.40% acetic acid
0.80% glu & 4.04% xyl-1%cCSL
Qp = 0.54 g/L/h
EtOH Yield = .48
0.75% acetic acid
0.79% glu & 4.05% xyl-1%cCSL
Qp = 0.48 g/L/h
EtOH Yield = .47
0.97% acetic acid
0.81% glu & 4.05% xyl-1%cCSL
Qp = 0.31 g/L/h
EtOH Yield = .48
0.40% acetic acid
0.78% glu & 4.01% xyl-.25%cCSL + N
Qp = 0.62 g/L/h
EtOH Yield =.49

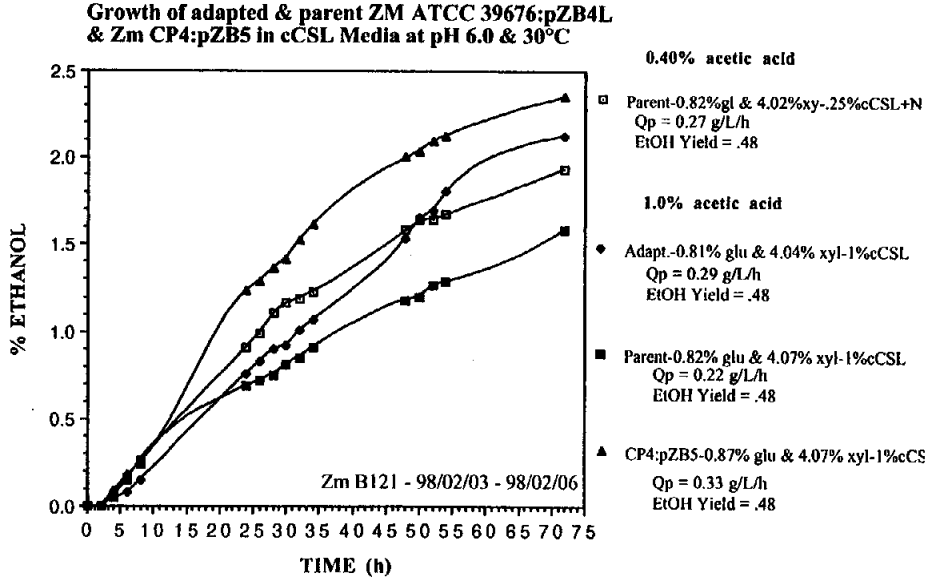

Zm B120 - 98/01/27 - 98/01/29
O.D. 600 nm.
TIME (h)
0.40% acetic acid
Parent-0.80% glu & 4.07% xyl ZM
Biomass = 0.88 g/L
Y x/s = .025
Adapt.-0.83% glu & 4.06% xyl ZM
Biomass = 0.87 g/L
Y x/s = .019
0.75% acetic acid
Parent-0.81% glu & 4.02% xyl ZM
Biomass = 0.70 g/L
Y x/s = .021
Adapt.-0.83% glu & 4.03% xyl ZM
Biomass = 0.79 g/L
Y x/s = .022
0.0% acetic acid
Parent-0.83% glu & 4.04% xyl ZM
Biomass = 1.38 g/L
Y x/s = .035
Adapt.-0.87% glu & 4.03% xyl ZM
Biomass = 1.34 g/L
Y x/s = .032

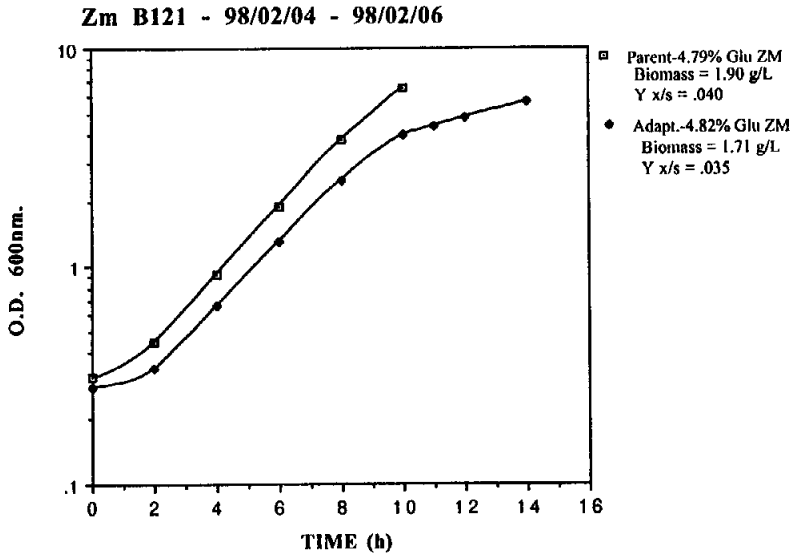

Growth of parent & adapted ATCC 39676:pZB4L
in ZM at pH 5.75 or 6.0 & 30°C +/- acetic acid
Zm B120 - 98/01/27 - 98/01/29
% GLUCOSE or % XYLOSE
TIME (h)
0.40% acetic acid
Parent-0.80% Glucose & 4.07% xylose ZM
Qsg = 1.33 g/L/h
Qsx = 0.90 g/L/h
Adapt.-0.83% Glucose & 4.06% xylose ZM
Qsg = 1.36 g/L/h
Qsx = 1.19 g/L/h
0.75% acetic acid
Parent-0.81% Glucose & 4.02% xylose ZM
Qsg = 1.01 g/L/h
Qsx = 0.84 g/L/h
Adapt.-0.83% Glucose & 4.03% xylose ZM
Qsg = 1.04 g/L/h
Qsx = 1.12 g/L/h
0.0% acetic acid
Parent-0.83% glucose & 4.04% xylose ZM
Qsx = 1.35 g/L/h
Adapt.-0.87% Glucose & 4.03% xylose ZM
Qsx = 1.55 g/L/h

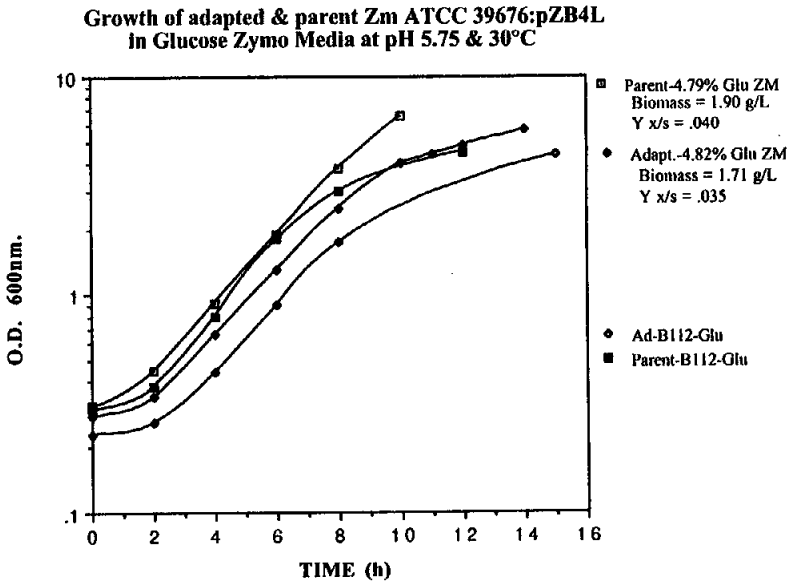

Growth of parent & adapted ATCC 39676:pZB4L
in ZM at pH 5.75 or 6.0 & 30°C +/- acetic acid
% ETHANOL
TIME (h)
Zm B120 - 98/01/27 - 98/01/29
0.40% acetic acid
Parent-0.80% glu & 4.07% xyl ZM
Qp = 0.52 g/L/h
EtOH Yield = .48
Adapt.-0.83% glu & 4.06% xyl ZM
Qp = 0.69 g/L/h
EtOH Yield = .48
0.75% acetic acid
Parent-0.81% glu & 4.02% xyl ZM
Qp = 0.49 g/L/h
EtOH Yield = .48
Adapt.-0.83% glu & 4.03% xyl ZM
Qp = 0.65 g/L/h
EtOH Yield = .48
0.0% acetic acid
Parent-0.83% glu & 4.04% xyl ZM
Qp = 0.79 g/L/h
EtOH Yield = .48
Adapt.-0.87% glu & 4.03% xyl ZM
Qp = 0.90 g/L/h
EtOH Yield = .48

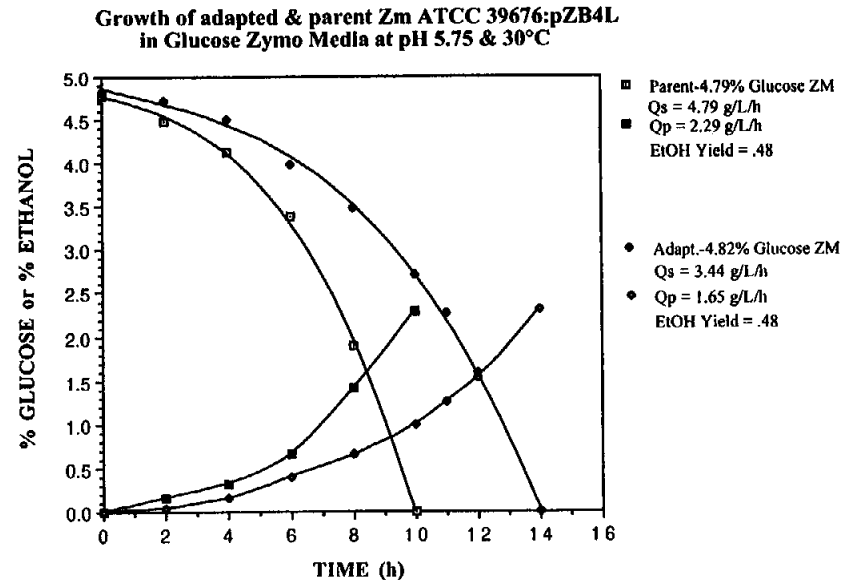

Zm B121 - 98/02/03 - 98/02/06
O.D. 600 nm.
10
1
.1
0 5 10 15 20 25 30 35 40 45 50 55 60 65 70 75
TIME (h)
0.40% acetic acid
Par-0.82%gl&4.02%xy-.25%cCSL+N
Biomass = 0.61 g/L
Yx/s = .030
1.0% acetic acid
Adapt-0.81% glu & 4.04% xyl-1%cCSL
Biomass = 0.72 g/L
Y x/s = .022
Parent-0.82% glu & 4.07% xyl-1%cCSL
Biomass = 0.63g/L
Y x/s = .025
CP4:pZB5-0.87%glu &4.07%xyl-1%cCSL
Biomass = 0.74 g/L
Y x/s = .018

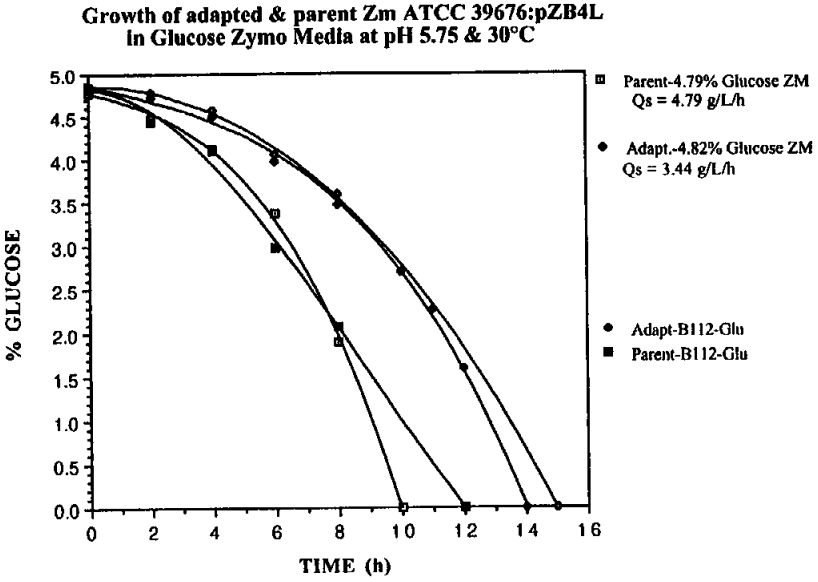

Growth of adapted & parent ZM ATCC 39676:pZB4L
& Zm CP4:pZB5 In cCSL Media at pH 6.0 & 30°C
Zm B121 - 98/02/03 - 98/02/06
% GLUCOSE or % XYLOSE
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
0 5 10 15 20 25 30 35 40 45 50 55 60 65 70 75
TIME (h)
0.40% acetic acid
Parent-0.82% glu &
4.02% xyl-.25% cCSL +N
Qsg = 0.82 g/L/h
Qsx = 0.44 g/L/h
1.0% acetic acid
Adapt-0.81% glucose &
4.04% xyl-1% cCSL
Qsg = 0.58 g/L/h
Qsx = 0.51 g/L/h
Parent-0.82% glucose &
4.07% xyl-1% cCSL
Qsg = 0.59 g/L/h
Qsx = 0.34 g/L/h
CP4:pZB5-0.87% glucose &
4.07% xyl-1% cCSL
Qsg = 0.62 g/L/h
Qsx = 0.56 g/L/h

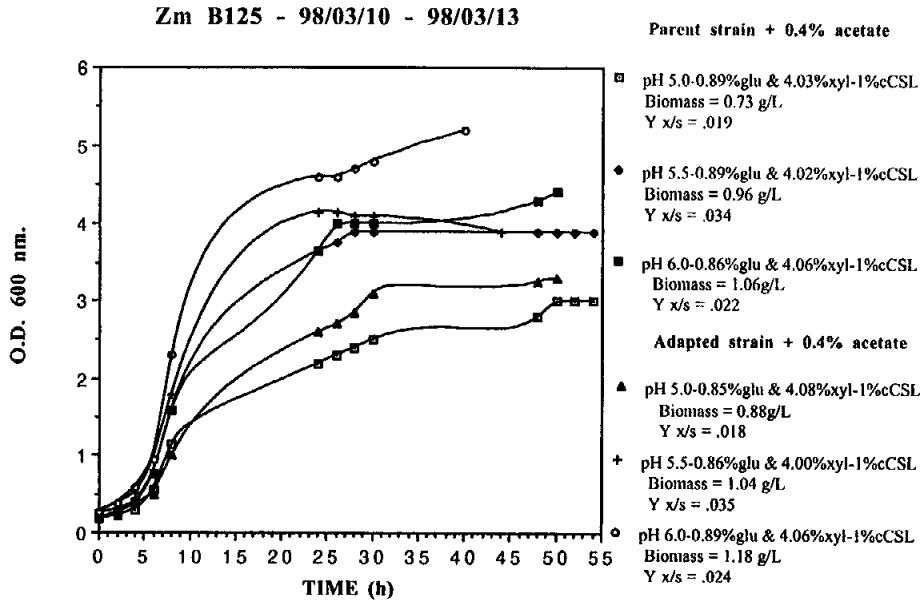

Growth of adapted & parent ZM ATCC 39676:pZB4L
& Zm CP4:pZB5 in cCSL Media at pH 6.0 & 30°C
% ETHANOL
2.5
2.0
1.5
1.0
0.5
0.0
0 5 10 15 20 25 30 35 40 45 50 55 60 65 70 75
TIME (h)
Zm B121 - 98/02/03 - 98/02/06
0.40% acetic acid
Parent-0.82%gl & 4.02%xy-.25%cCSL+N
Qp = 0.27 g/L/h
EtOH Yield = .48
1.0% acetic acid
Adapt.-0.81% glu & 4.04% xyl-1%cCSL
Qp = 0.29 g/L/h
EtOH Yield = .48
Parent-0.82% glu & 4.07% xyl-1%cCSL
Qp = 0.22 g/L/h
EtOH Yield = .48
CP4:pZB5-0.87% glu & 4.07% xyl-1%cCS
Qp = 0.33 g/L/h
EtOH Yield = .48

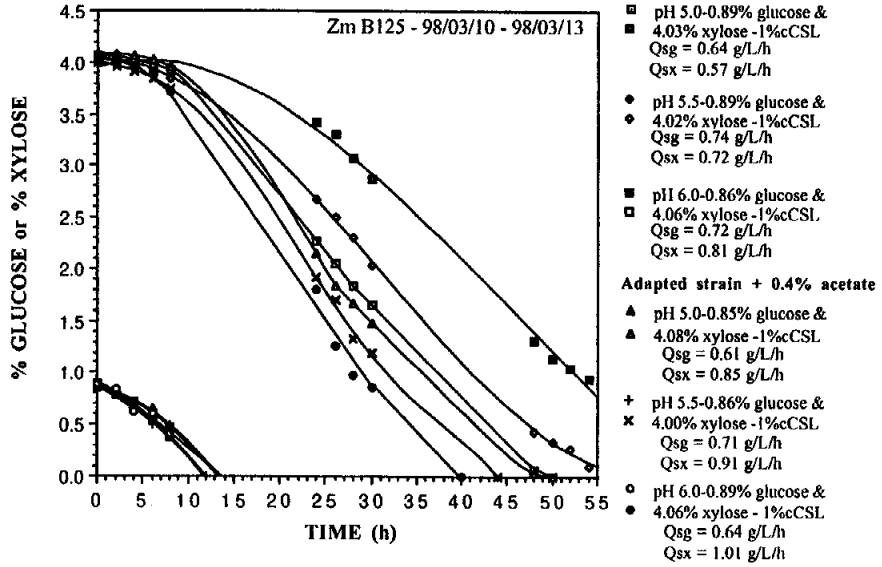

Zm B121 - 98/02/04 - 98/02/06
O.D. 600nm.
TIME (h)
Parent-4.79% Glu ZM
Biomass = 1.90 g/L
Y x/s = .040
Adapt.-4.82% Glu ZM
Biomass = 1.71 g/L
Y x/s = .035

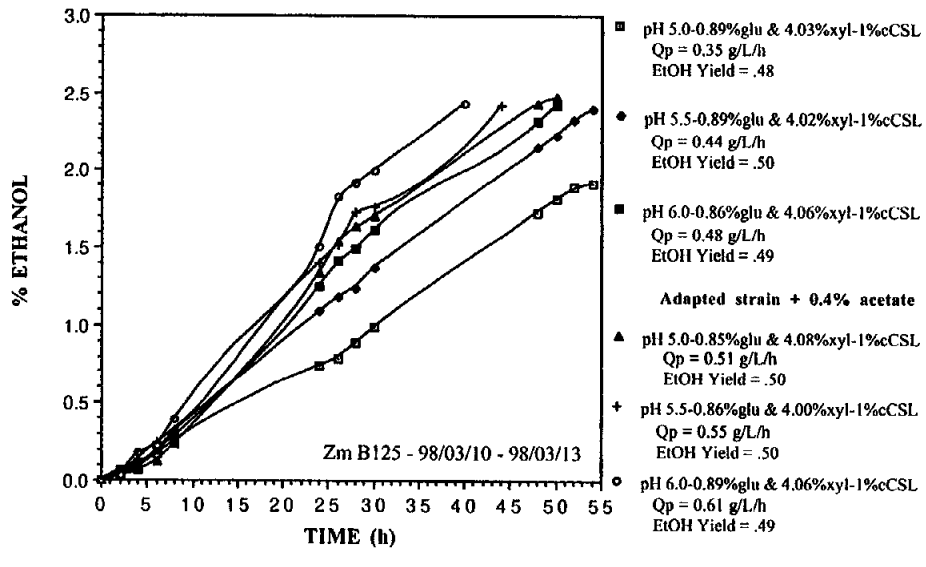

Growth of adapted & parent Zm ATCC 39676:pZB4L
in Glucose Zymo Media at pH 5.75 & 30°C
O.D. 600nm.
TIME (h)
Parent-4.79% Glu ZM
Biomass = 1.90 g/L
Y x/s = .040
Adapt.-4.82% Glu ZM
Biomass = 1.71 g/L
Y x/s = .035
Ad-B112-Glu
Parent-B112-Glu


Growth of adapted & parent Zm ATCC 39676:pZB4L
in Glucose Zymo Media at pH 5.75 & 30°C
% GLUCOSE or % ETHANOL
TIME (h)
Parent-4.79% Glucose ZM
Qs = 4.79 g/L/h
Qp = 2.29 g/L/h
EtOH Yield = .48
Adapt.-4.82% Glucose ZM
Qs = 3.44 g/L/h
Qp = 1.65 g/L/h
EtOH Yield = .48


Growth of adapted & parent Zm ATCC 39676:pZB4L
in Glucose Zymo Media at pH 5.75 & 30°C
% GLUCOSE
TIME (h)
Parent-4.79% Glucose ZM
Qs = 4.79 g/L/h
Adapt.-4.82% Glucose ZM
Qs = 3.44 g/L/h
Adapt-B112-Glu
Parent-B112-Glu


Zm B125 - 98/03/10 - 98/03/13
O.D. 600 nm.
TIME (h)
Parent strain + 0.4% acetate
pH 5.0-0.89%glu & 4.03%xyl-1%cCSL
Biomass = 0.73 g/L
Y x/s = .019
pH 5.5-0.89%glu & 4.02%xyl-1%cCSL
Biomass = 0.96 g/L
Y x/s = .034
pH 6.0-0.86%glu & 4.06%xyl-1%cCSL
Biomass = 1.06g/L
Y x/s = .022
Adapted strain + 0.4% acetate
pH 5.0-0.85%glu & 4.08%xyl-1%cCSL
Biomass = 0.88g/L
Y x/s = .018
pH 5.5-0.86%glu & 4.00%xyl-1%cCSL
Biomass = 1.04 g/L
Y x/s = .035
pH 6.0-0.89%glu & 4.06%xyl-1%cCSL
Biomass = 1.18 g/L
Y x/s = .024


Growth of parent & adapted ATCC 39676:pZB4L
in 1% cCSL at pH 5, 5.5 7 6 & 30°C
Zm B125 - 98/03/10 - 98/03/13
% GLUCOSE or % XYLOSE
TIME (h)
Parent strain + 0.4% acetate
pH 5.0-0.89% glucose &
4.03% xylose -1%cCSL
Qsg = 0.64 g/L/h
Qsx = 0.57 g/L/h
pH 5.5-0.89% glucose &
4.02% xylose -1%cCSL
Qsg = 0.74 g/L/h
Qsx = 0.72 g/L/h
pH 6.0-0.86% glucose &
4.06% xylose -1%cCSL
Qsg = 0.72 g/L/h
Qsx = 0.81 g/L/h
Adapted strain + 0.4% acetate
pH 5.0-0.85% glucose &
4.08% xylose -1%cCSL
Qsg = 0.61 g/L/h
Qsx = 0.85 g/L/h
pH 5.5-0.86% glucose &
4.00% xylose -1%cCSL
Qsg = 0.71 g/L/h
Qsx = 0.91 g/L/h
pH 6.0-0.89% glucose &
4.06% xylose - 1%cCSL
Qsg = 0.64 g/L/h
Qsx = 1.01 g/L/h


Growth of parent & adapted ATCC 39676:pZB4L
in 1% cCSL at pH 5, 5.5 7 6 & 30°C
Zm B125 - 98/03/10 - 98/03/13
% ETHANOL
TIME (h)
Parent strain + 0.4% acetate
pH 5.0-0.89%glu & 4.03%xyl-1%cCSL
Qp = 0.35 g/L/h
EtOH Yield = .48
pH 5.5-0.89%glu & 4.02%xyl-1%cCSL
Qp = 0.44 g/L/h
EtOH Yield = .50
pH 6.0-0.86%glu & 4.06%xyl-1%cCSL
Qp = 0.48 g/L/h
EtOH Yield = .49
Adapted strain + 0.4% acetate
pH 5.0-0.85%glu & 4.08%xyl-1%cCSL
Qp = 0.51 g/L/h
EtOH Yield = .50
pH 5.5-0.86%glu & 4.00%xyl-1%cCSL
Qp = 0.55 g/L/h
EtOH Yield = .50
pH 6.0-0.89%glu & 4.06%xyl-1%cCSL
Qp = 0.61 g/L/h
EtOH Yield = .49

APPENDIX E

Summaries of chemostat experiments for Task 3

C107- 98/01/28-98/02/13

OPERATIONAL PARAMETERS FOR CONTINUOUS FERMENTATIONS

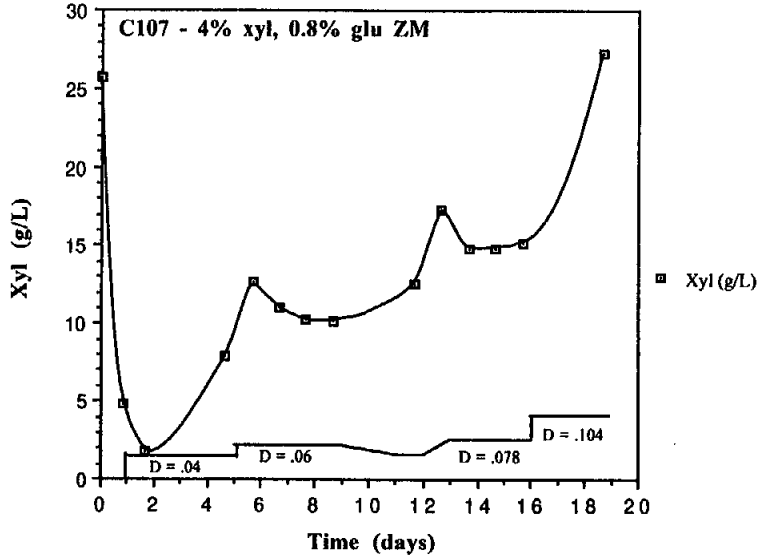
Sugar conversion by "adapted" *Zymomonas mobilis* ATCC 39676:pZB4L

		SUBSTRATE			[PRODUCTS]					PRODUCTIVITY			YIELD		
Date	pH	[Glu]	[Xyl]	Residual	Biomass	EtOH	Xylitol	Lactate	Acetate	D	Q_p	q_p	$Y_{p/s}$	Conversion	% C
		g/L	g/L	[Xyl] (g/L)	g/L	g/L	g/L?	g/L	g/L	hr^{-1}	g P/L/h	g P/g cell/h	g P/g Glu	Efffic (%)	Recovery
C107- Medium=ZM(5g/L YE + Zymos salts +.8g/L NH_4Cl) (flow started 20 h after inoculation)															
98/01/29	5.75	24.73	25.68	4.78	1.65	21.91	0.00	3.08	0.00	.000	-	-	.48	94	104
98/01/30	5.75	8.22	40.43	1.81	1.35	22.77	0.00	0.26	0.00	.040	0.91	0.67	.49	96	99
98/02/02	**5.75**	**8.22**	**40.43**	**7.86**	**1.23**	**19.00**	**0.00**	**2.38**	**0.00**	**.040**	**0.76**	**0.62**	**.47**	**92**	**100**
98/02/03	5.75	7.29	35.66	12.68	0.90	14.15	0.05	2.08	0.00	.059	0.83	0.93	.47	92	101
98/02/04	5.75	7.29	35.66	11.02	1.11	15.07	0.24	1.38	0.00	.059	0.89	0.80	.47	92	101
98/02/05	**5.75**	**7.29**	**35.66**	**10.26**	**1.02**	**15.69**	**0.60**	**1.80**	**0.00**	**.059**	**0.93**	**0.91**	**.48**	**94**	**104**
98/02/06	**5.75**	**7.29**	**36.66**	**10.20**	**1.08**	**15.28**	**0.00**	**1.70**	**0.00**	**.059**	**0.90**	**0.83**	**.47**	**92**	**100**
98/02/09	5.75	8.72	40.37	12.51	1.05	17.45	0.00	1.11	0.00	.040	0.70	0.66	.48	94	100
98/02/10	5.75	8.72	40.37	17.33	1.05	15.02	0.00	2.30	0.00	.060	0.90	0.86	.47	92	102
98/02/11	5.75	8.72	40.37	14.81	0.90	16.55	0.00	1.51	0.00	.078	1.29	1.43	.48	94	101
98/02/12	**5.75**	**8.72**	**40.37**	**14.87**	**1.02**	**16.31**	**0.00**	**1.02**	**0.00**	**.078**	**1.27**	**1.25**	**.48**	**94**	**100**
98/02/13	5.75	8.72	40.37	15.18	1.02	16.24	0.00	0.77	0.00	.080	1.30	1.27	.48	94	100
98/02/16	**5.75**	**8.40**	**40.30**	**27.28**	**1.10**	**10.12**	**0.00**	**1.17**	**0.00**	**.104**	**1.05**	**0.96**	**.47**	**92**	**102**

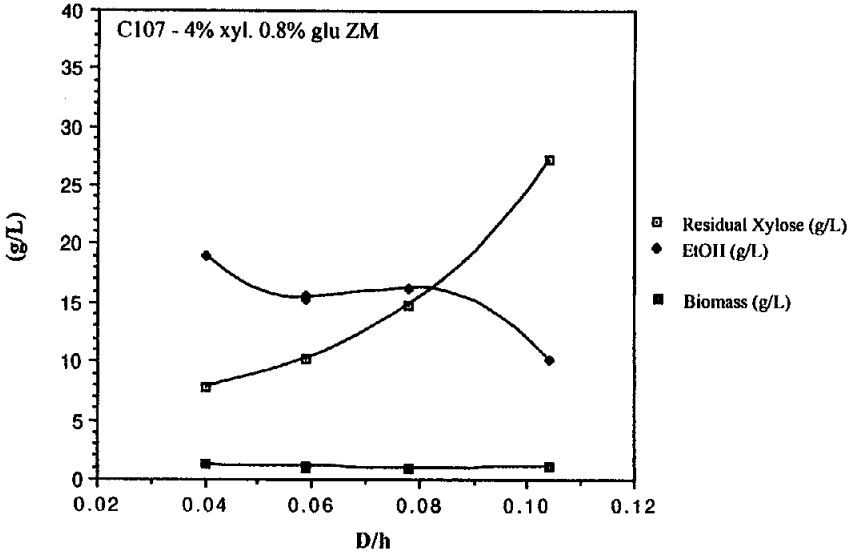
-terminated experiment

* Biomass measured by filter method;
only lines in **bold** are at steady state
? unknown presumed to be xylitol

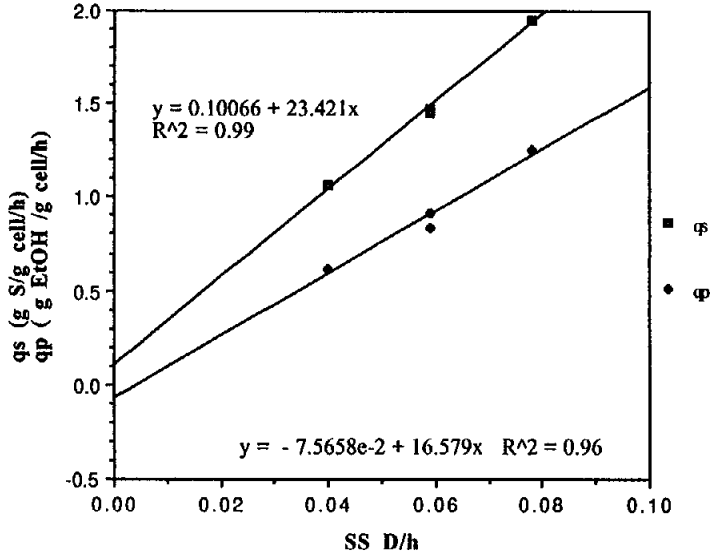
"Adapted" ATCC 39676:pZB4L in ZM
pH 5.75 & 30°C



Continuous culture of "adapted" ATCC 396767:pZB4L
in Zymo Media at pH 5.75 & 30°C



C107 - 4% xylose, 0.8% glucose, ZM



C109- 98/02/17-98/03/11

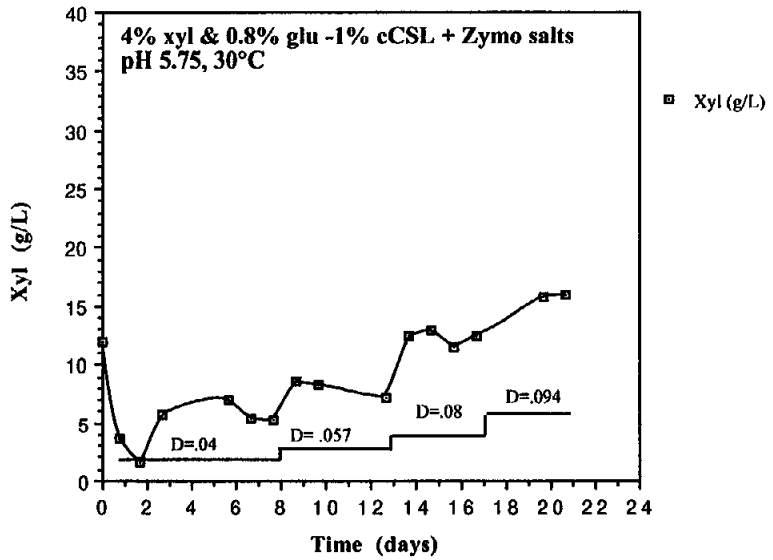
OPERATIONAL PARAMETERS FOR CONTINUOUS FERMENTATIONS

Sugar conversion by "adapted" *Zymomonas mobilis* ATCC 39676:pZB4L

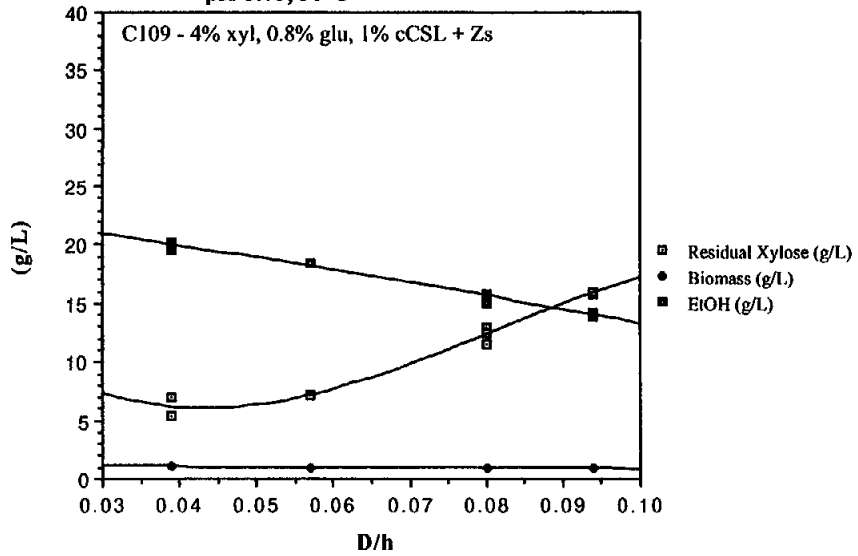
		SUBSTRATE			[PRODUCTS]					PRODUCTIVITY			YIELD		
Date	pH	[Glu] g/L	[Xyl] g/L	Residual [Xyl] (g/L)	Biomass g/L	EtOH g/L	Xylitol g/L?	Lactate g/L	Acetate g/L	D hr^{-1}	Q_p g P/L/h	q_p g P/g cell/h	$Y_{p/s}$ g P/g Glu	Conversion Efffic (%)	% C Recovery
C109 - Medium=1%cCSL + Zymos salts (flow started 18 h after inoculation)															
98/02/18	5.75	24.03	11.95	3.63	0.93	16.18	0.00	0.02	0.00	.000	-	-	.50	98	101
98/02/19	5.75	7.83	40.05	1.56	1.29	21.18	0.00	2.93	0.00	.040	0.85	0.66	.46	90	99
98/02/20	5.75	7.83	40.05	5.74	1.38	19.91	0.00	2.87	0.00	.040	0.80	0.58	.47	92	103
98/02/23	**5.75**	**7.83**	**40.05**	**6.94**	**1.05***	**19.62**	**0.00**	**2.89**	**0.00**	**.039**	**0.77**	**0.73**	**.48**	**94**	**103**
98/02/24	**5.75**	**7.83**	**40.05**	**5.36**	**1.05**	**20.29**	**0.89**	**2.38**	**0.00**	**.039**	**0.79**	**0.75**	**.48**	**94**	**103**
98/02/25	5.75	7.57	39.01	5.33	1.14	19.14	0.59	2.85	0.00	.045	0.86	0.76	.46	90	102
98/02/26	5.75	7.57	39.01	8.55	1.26	17.31	0.79	2.09	0.00	.058	1.00	0.80	.46	90	102
98/02/27	5.75	7.57	39.01	8.26	0.90	18.11	0.28	2.28	0.00	.057	1.03	1.15	.47	92	102
98/03/02	**5.75**	**7.57**	**39.01**	**7.14**	**0.96**	**18.49**	**0.28**	**2.34**	**0.00**	**.057**	**1.05**	**1.10**	**.47**	**92**	**101**
98/03/03	5.75	7.57	39.01	12.39	0.96	16.04	0.00	2.43	0.00	.080	1.28	1.34	.47	92	102
98/03/04	**5.7**	**7.57**	**39.01**	**12.89**	**0.91**	**15.05**	**0.89**	**2.30**	**0.00**	**.080**	**1.20**	**1.32**	**.45**	**88**	**100**
98/03/05	**5.75**	**7.77**	**39.37**	**11.55**	**0.99**	**15.75**	**0.89**	**2.53**	**0.00**	**.080**	**1.26**	**1.27**	**.44**	**86**	**100**
98/03/06	**5.75**	**7.77**	**39.37**	**12.36**	**0.99**	**15.69**	**0.31**	**2.39**	**0.00**	**.080**	**1.26**	**1.27**	**.45**	**88**	**100**
98/03/09	**5.75**	**7.77**	**39.37**	**15.76**	**0.90**	**13.91**	**0.90**	**1.75**	**0.00**	**.094**	**1.31**	**1.45**	**.44**	**86**	**102**
98/03/10	**5.75**	**7.77**	**39.37**	**15.96**	**0.91**	**14.20**	**0.48**	**2.40**	**0.00**	**.094**	**1.33**	**1.47**	**.46**	**90**	**101**
98/03/11 - terminated experiment															

* Biomass measured by filter method;
only lines in **bold** are at steady state
? unknown presumed to be xylitol

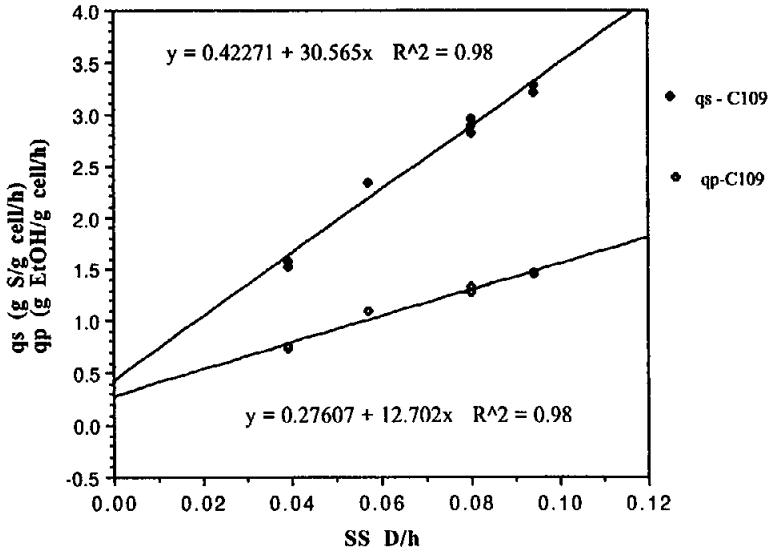
"adapted" ATCC 39676:pZB4L



"Adapted" ATCC 39676:pZB4L
in 1% CCSL + Zymo salts
pH 5.75, 30°C



C109 - 4% xyl/0.8% glu - 1% (v/v) cCSL + Zymo salts



C110- 98/02/17-98/02/20

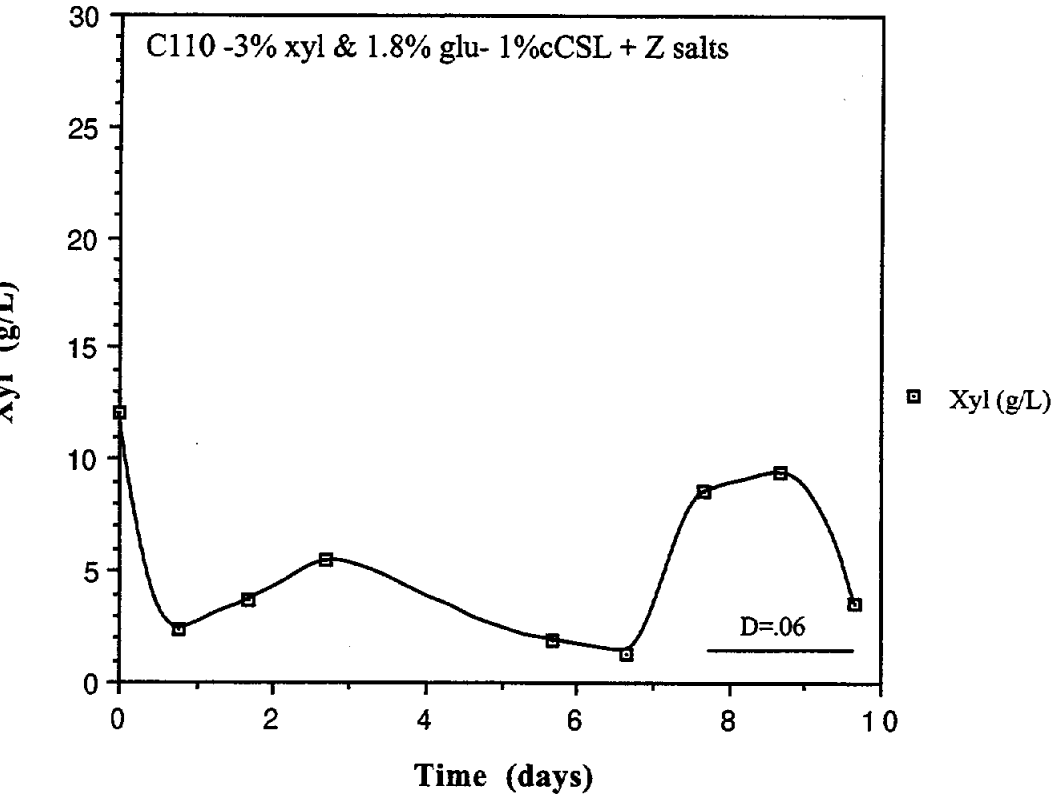
OPERATIONAL PARAMETERS FOR CONTINUOUS FERMENTATIONS

Sugar conversion by "adapted" *Zymomonas mobilis* ATCC 39676:pZB4L

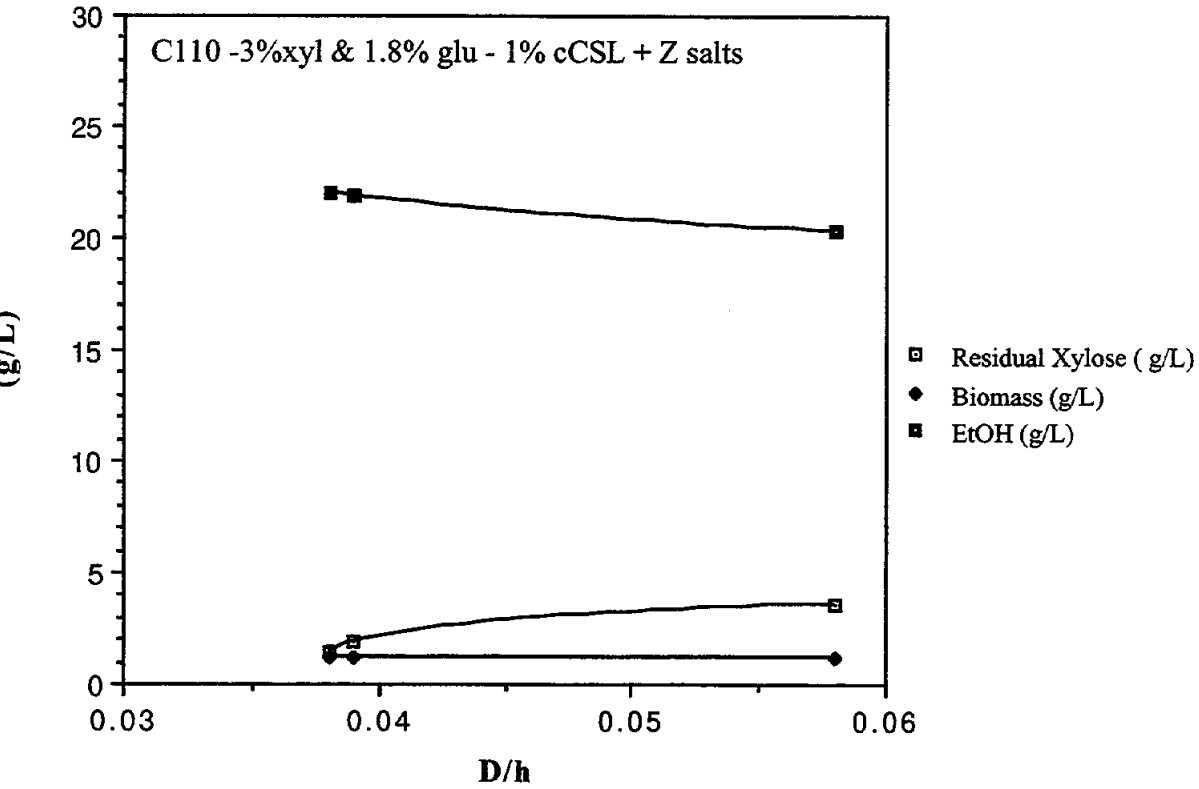
		SUBSTRATE			[PRODUCTS]					PRODUCTIVITY			YIELD		
Date	pH	[Glu] g/L	[Xyl] g/L	Residual [Xyl] (g/L)	Biomass g/L	EtOH g/L	Xylitol g/L?	Lactate g/L	Acetate g/L	D hr^{-1}	Q_p g P/L/h	q_p g P/g cell/h	$Y_{p/s}$ g P/g Glu	Conversion Efffic (%)	% C Recovery
C110 - Medium=1%cCSL + Zymos salts (flow started 18 h after inoculation)															
98/02/18	5.75	26.08	12.05	2.37	1.11	17.81	0.00	0.04	0.00	.000	-	-	.50	98	101
98/02/19	5.75	18.95	30.47	3.70	1.41	19.92	0.00	4.80	0.00	.039	0.78	0.55	.44	86	100
98/02/20	5.75	18.95	30.47	5.45	1.44	20.19	0.00	3.08	0.00	.039	0.78	0.55	.46	90	101
98/02/23	**5.75**	**18.95**	**30.47**	**1.90**	**1.17***	**21.89**	**0.26**	**2.91**	**0.00**	**.039**	**0.85**	**0.73**	**.46**	**90**	**100**
98/02/24	**5.75**	**18.95**	**30.47**	**1.37**	**1.23**	**22.02**	**1.31**	**2.91**	**0.00**	**.038**	**0.84**	**0.68**	**.46**	**90**	**101**
98/02/25	5.75	17.54	30.13	8.64	1.23	17.44	1.58	3.42	0.00	.044	0.77	0.62	.45	88	103
98/02/26	5.75	17.54	30.13	9.41	1.11	17.54	0.36	2.48	0.00	.063	1.11	0.78	.46	90	101
98/02/27	**5.75**	**17.54**	**30.13**	**3.62**	**1.14**	**20.33**	**0.65**	**2.40**	**0.00**	**.058**	**1.18**	**1.03**	**.46**	**90**	**100**
98/03/02- pH controller failed - experiment terminated															

* Biomass measured by filter method;
only lines in **bold** are at steady state
? unknown presumed to be xylitol

"adapted" ATCC 39676:pZB4L
at pH 5.75 & 30°C



"adapted" ATCC 39676:pZB4L
at pH 5.75 & 30°C



C111- 98/03/03-98/03/13

OPERATIONAL PARAMETERS FOR CONTINUOUS FERMENTATIONS

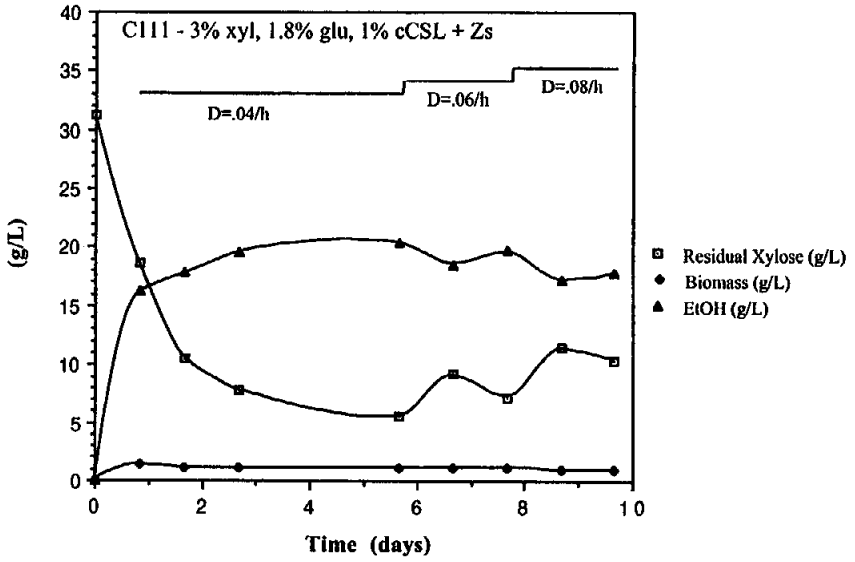
Sugar conversion by "adapted" *Zymomonas mobilis* ATCC 39676:pZB4L

		SUBSTRATE			[PRODUCTS]					PRODUCTIVITY			YIELD		
Date	pH	[Glu] g/L	[Xyl] g/L	Residual [Xyl] (g/L)	Biomass g/L	EtOH g/L	Xylitol g/L?	Lactate g/L	Acetate g/L	D hr^{-1}	Q_p g P/L/h	q_p g P/g cell/h	$Y_{p/s}$ g P/g Glu	Conversion Efffic (%)	% C Recovery
C111- Medium=1%cCSL + Zymos salts (flow started 20h after inoculation)															
98/03/04	5.75	26.44	31.30	18.63	1.44	16.27	0.00	0.50	0.00	.000	-	-	.50	98	103
98/03/05	5.75	18.56	30.12	10.52	1.10	17.80	0.00	2.79	0.00	.040	0.71	0.65	.47	92	102
98/03/06	5.75	18.56	30.12	7.87	1.05	19.63	0.47	1.67	0.00	.040	0.78	0.75	.48	94	102
98/03/09	**5.75**	**18.56**	**30.12**	**5.60**	**1.05**	**20.38**	**0.23**	**2.14**	**0.00**	**.040**	**0.82**	**0.78**	**.47**	**92**	**101**
98/03/10	5.75	18.56	30.12	9.21	1.05	18.55	0.41	1.48	0.00	.059	1.09	1.04	.47	92	100
98/03/11	**5.75**	**18.56**	**30.12**	**7.18**	**1.14**	**19.73**	**0.88**	**2.37**	**0.00**	**.057**	**1.12**	**0.99**	**.48**	**94**	**103**
98/03/12	5.75	17.69	30.09	11.43	0.95	17.25	0.68	1.26	0.00	.083	1.43	1.51	.47	92	101
98/03/13	**5.75**	**17.69**	**30.09**	**10.43**	**0.95**	**17.80**	**0.59**	**1.34**	**0.00**	**.080**	**1.42**	**1.50**	**.48**	**94**	**101**

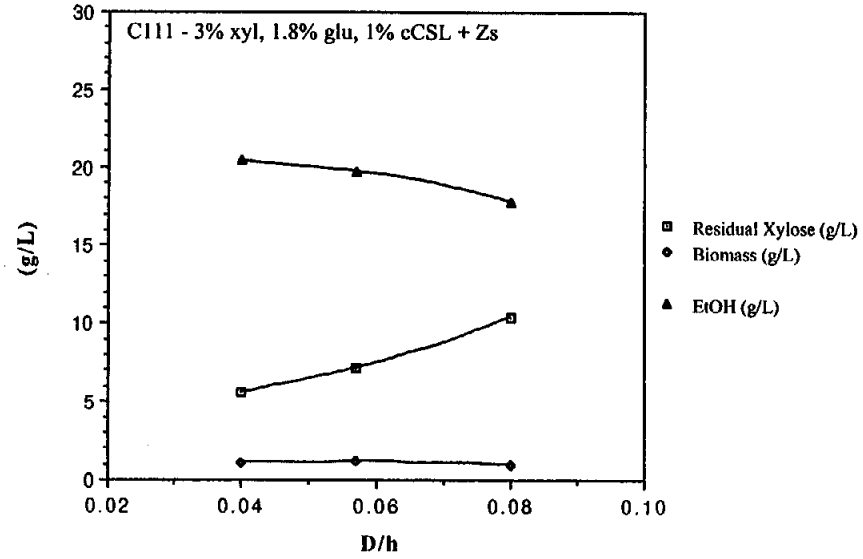
-terminated experiment

* Biomass measured by filter method;
only lines in **bold** are at steady state
? unknown presumed to be xylitol

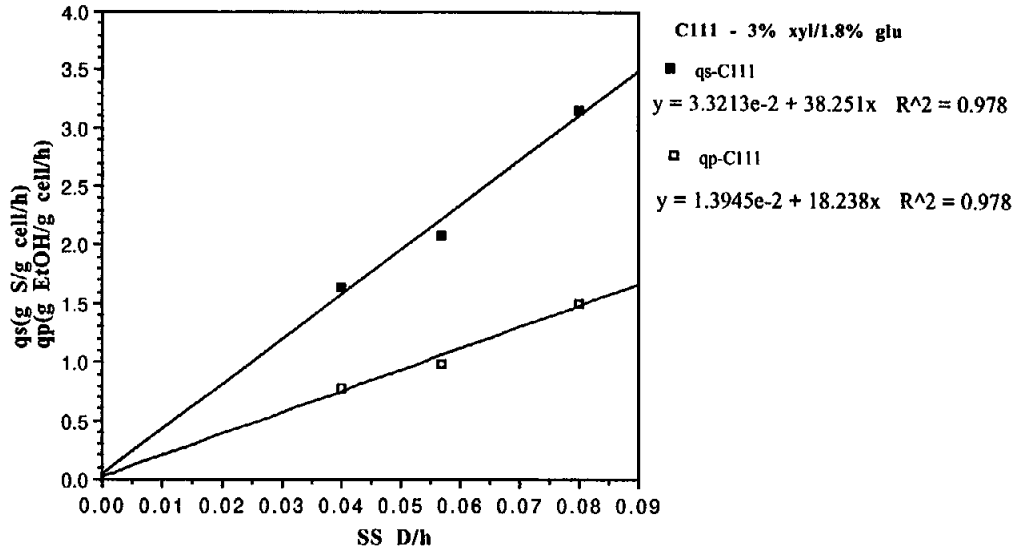
Growth of "adapted" ATCC 39676:pZB4L
at pH 5.75 & 30°C



adapted ATCC 39676:pZB4L
in 1% CCSL + Zymo salts
pH 5.75, 30°C



Continuous culture of "adapted" Zm ATCC 39676:pZB4L



C112- 98/03/26-98/04/30

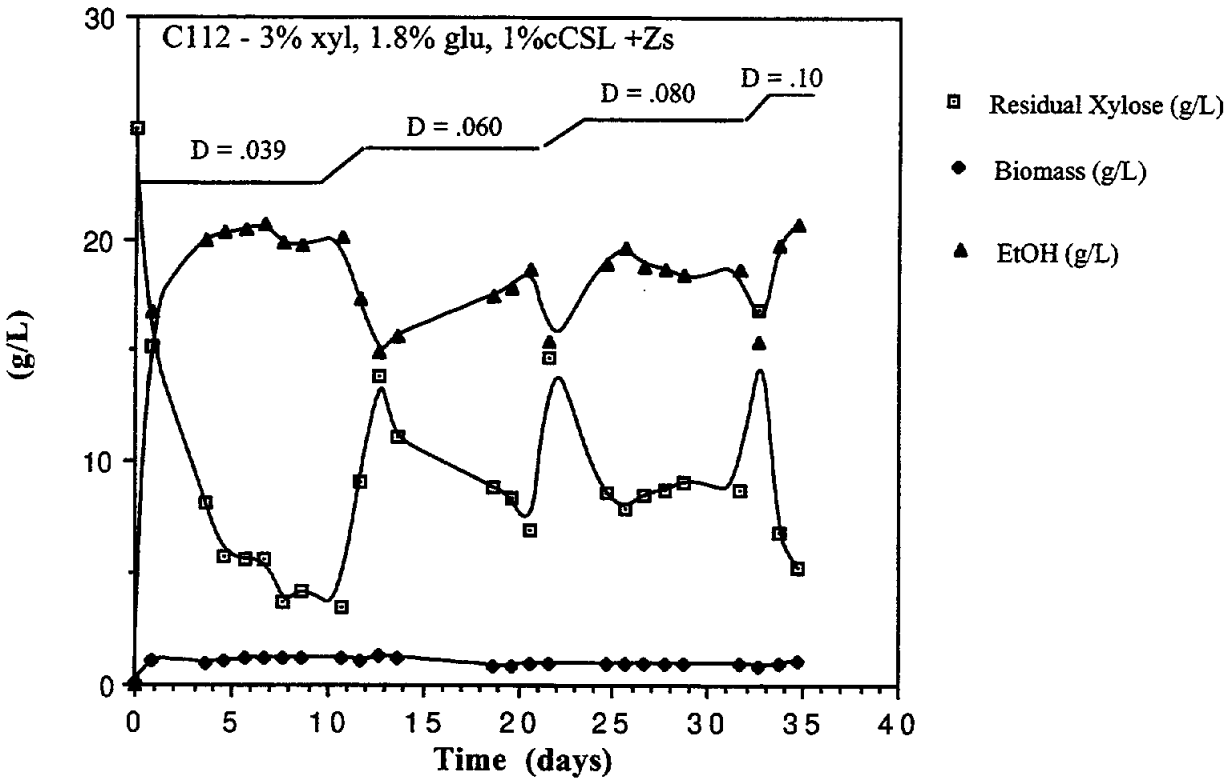
OPERATIONAL PARAMETERS FOR CONTINUOUS FERMENTATIONS

Sugar conversion by "adapted" *Zymomonas mobilis* ATCC 39676:pZB4L

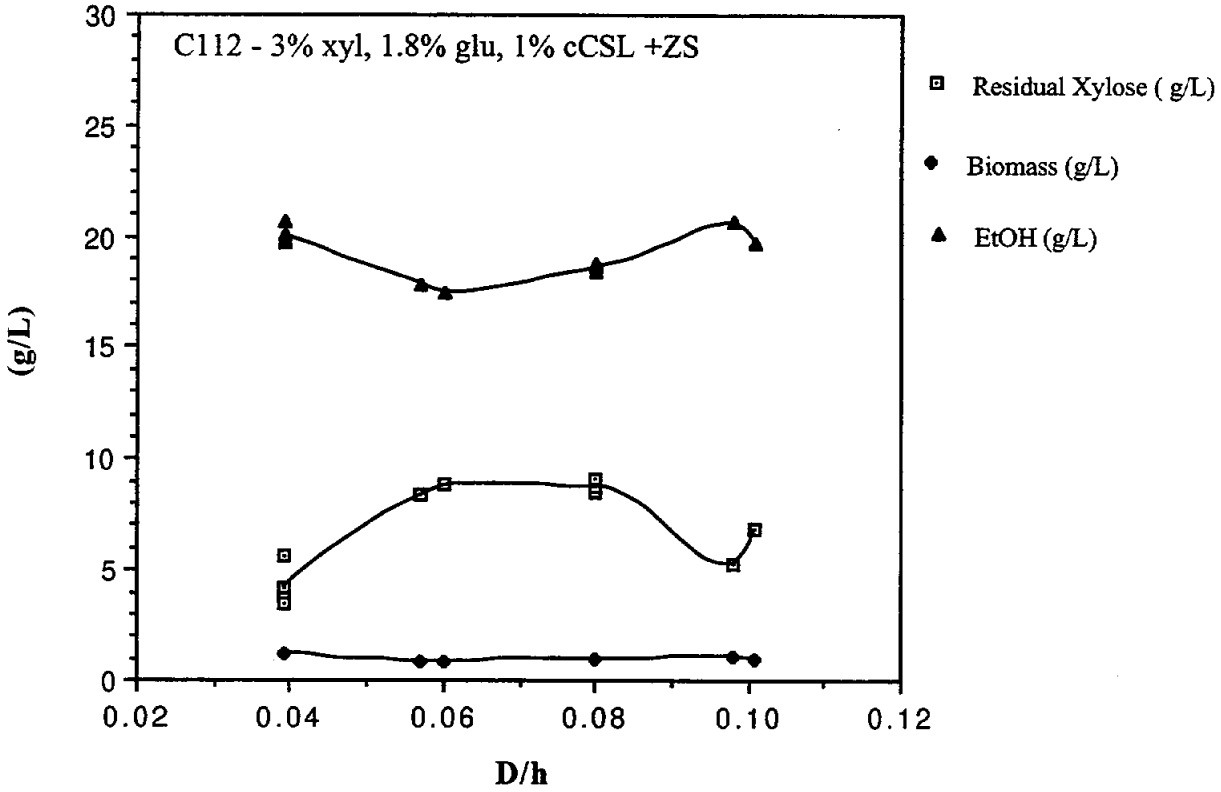
		SUBSTRATE			[PRODUCTS]					PRODUCTIVITY			YIELD		
Date	pH	[Glu] g/L	[Xyl] g/L	Residual [Xyl] (g/L)	Biomass g/L	EtOH g/L	Xylitol g/L?	Lactate g/L	Acetate g/L	D hr^{-1}	Q_p g P/L/h	q_p g P/g cell/h	$Y_{p/s}$ g P/g Glu	Conversion Efffic (%)	% C Recovery
C112- Medium=1%cCSL + Zymos salts (flow started 20h after inoculation)															
98/03/27	5.75	25.0	25.0	15.20	1.11	16.75	0.00	0.43	0.00	.000	-	-	.48	94	99
98/03/30	5.75	18.34	31.00	8.18	1.01	19.96	0.00	0.56	0.00	.039	0.78	0.77	.48	94	99
98/03/31	5.75	18.34	31.00	5.75	1.05	20.31	0.00	2.40	0.00	.039	0.79	0.75	.47	92	100
98/04/01	5.75	18.34	31.00	5.61	1.16*	20.42	0.00	2.25	0.00	.039	0.80	0.69	.47	92	100
98/04/02	**5.75**	**18.34**	**31.00**	**5.67**	**1.25***	**20.70**	**0.00**	**2.36**	**0.00**	**.039**	**0.81**	**0.65**	**.47**	**92**	**101**
98/04/03	**5.75**	**16.90**	**29.23**	**3.66**	**1.25**	**19.80**	**0.00**	**2.34**	**0.00**	**.039**	**0.77**	**0.62**	**.47**	**92**	**100**
98/04/04	**5.75**	**16.90**	**29.23**	**4.20**	**1.25**	**19.68**	**0.00**	**2.09**	**0.00**	**.039**	**0.77**	**0.61**	**.47**	**92**	**100**
98/04/06	**5.75**	**16.90**	**29.23**	**3.42**	**1.25**	**20.11**	**0.00**	**2.10**	**0.00**	**.039**	**0.78**	**0.63**	**.47**	**92**	**101**
98/04/07	5.75	16.90	29.23	9.03	1.04	17.38	0.00	2.04	0.00	.062	1.08	1.04	.47	92	100
98/04/08	5.75	16.90	29.23	13.86	1.27	14.89	0.00	1.81	0.00	.060	0.89	0.70	.46	90	100
98/04/09	5.75	16.90	29.23	11.08	1.24	15.69	0.00	1.81	0.00	.060	0.94	0.76	.45	88	98
98/04/14	**5.75**	**17.40**	**29.41**	**8.86**	**0.87***	**17.45**	**0.00**	**1.81**	**0.00**	**.060**	**1.05**	**1.20**	**.46**	**90**	**98**
98/04/15	**5.75**	**17.40**	**29.41**	**8.37**	**0.87**	**17.81**	**0.00**	**2.38**	**0.00**	**.057**	**1.02**	**1.17**	**.46**	**90**	**100**
98/04/16	5.75	17.40	29.41	6.89	0.99	18.63	0.00	2.00	0.00	.080	1.49	1.51	.47	92	99
98/04/17	5.75	18.14	29.68	14.68	0.99	15.38	0.00	1.29	0.00	.080	1.23	1.24	.46	90	99
98/04/20	5.00	18.14	29.68	8.60	1.01	18.94	0.00	0.92	0.00	.080	1.52	1.50	.48	94	99
98/04/21	5.75	18.29	30.34	7.89	0.99	19.59	0.00	1.72	0.00	.080	1.57	1.58	.48	94	101
98/04/22	**5.75**	**18.29**	**30.34**	**8.51**	**0.99***	**18.81**	**0.00**	**1.35**	**0.00**	**.080**	**1.50**	**1.52**	**.47**	**92**	**98**
98/04/23	**5.75**	**18.29**	**30.34**	**8.69**	**0.99**	**18.66**	**0.00**	**1.64**	**0.00**	**.080**	**1.49**	**1.51**	**.47**	**92**	**99**
98/04/24	**5.75**	**18.29**	**30.34**	**9.07**	**0.99**	**18.35**	**0.00**	**1.86**	**0.00**	**.080**	**1.47**	**1.48**	**.46**	**90**	**99**
98/04/27	5.75	18.29	30.34	8.76	0.99	18.64	0.00	1.72	0.00	.089	1.66	1.68	.47	92	99
98/04/28	5.75	18.29	30.34	16.82	0.86	15.47	0.00	1.77	0.00	.100	1.55	1.80	.48	94	103
98/04/29	**5.75**	**18.29**	**30.34**	**6.82**	**0.92***	**19.67**	**0.00**	**0.76**	**0.00**	**.101**	**1.99**	**2.16**	**.47**	**92**	**97**
98/04/30	**5.75**	**18.29**	**30.34**	**5.25**	**1.04**	**20.64**	**0.00**	**0.21**	**0.00**	**.098**	**2.02**	**1.94**	**.48**	**94**	**97**

-* Biomass measured by filter method;
-only lines in **bold** are at steady state

Continuous culture of "adapted" ATCC 39676:pZB4L in 1% cCSL + Zymo salts (or tap water) at pH 5.75 & 30°C



Continuous culture of "adapted" ATCC 39676:pZB4L in 1% cCSL + Zymo salts (or tap water) at pH 5.75 & 30°C



C122- 98/07/22 - 98/08/04

OPERATIONAL PARAMETERS FOR CONTINUOUS FERMENTATIONS

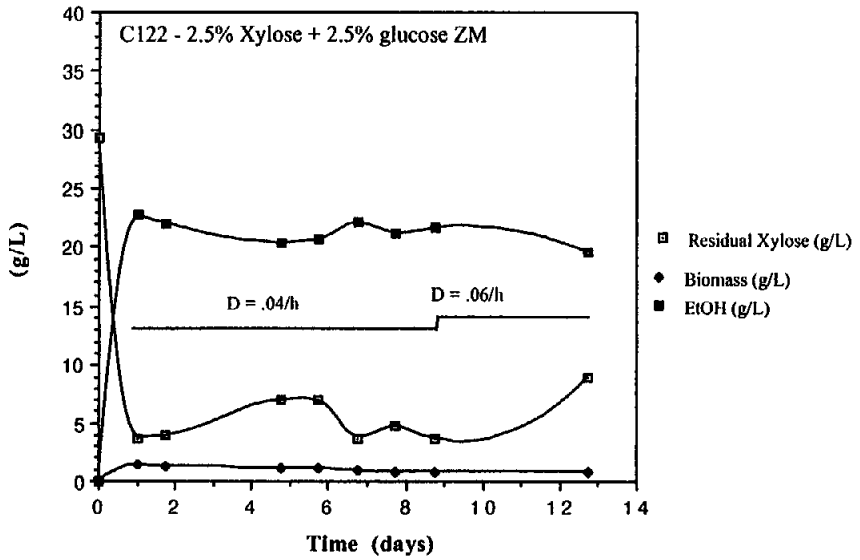
Sugar conversion by "adapted" *Zymomonas mobilis* ATCC 39676:pZB4L

		SUBSTRATE			[PRODUCTS]					PRODUCTIVITY			YIELD		
Date	pH	[Glu] g/L	[Xyl] g/L	Residual [Xyl] (g/L)	Biomass g/L	EtOH g/L	Xylitol g/L?	Lactate g/L	Acetate g/L	D hr^{-1}	Q_p g P/L/h	q_p g P/g cell/h	$Y_{p/s}$ g P/g Glu	Conversion Efffic (%)	% C Recovery
C122- Medium=ZM(flow started 24h after inoculation)															
98/07/23	5.75	20.94	29.40	3.72	1.38*	22.80	0.05	0.24	0.00	.000	-	-	.49	96	100
98/07/24	5.75	24.40	25.87	3.94	1.26	22.02	0.00	0.24	0.00	.039	0.86	0.68	.48	94	97
98/07/27	5.75	24.40	25.87	7.02	1.14	20.44	0.00	3.48	0.00	.039	0.80	0.70	.47	92	103
98/07/28	5.75	24.40	25.87	7.07	1.08	20.66	0.00	3.04	0.00	.040	0.83	0.77	.48	94	103
98/07/29	**5.75**	**24.40**	**25.87**	**3.73**	**0.90**	**22.19**	**0.00**	**3.11**	**0.00**	**.040**	**0.89**	**0.99**	**.48**	**94**	**102**
98/07/30	**5.75**	**24.40**	**25.87**	**4.78**	**0.78***	**21.16**	**0.00**	**3.84**	**0.00**	**.040**	**0.85**	**1.09**	**.47**	**92**	**101**
98/07/31	**5.75**	**24.40**	**25.87**	**3.65**	**0.78**	**21.67**	**0.00**	**3.46**	**0.00**	**.039**	**0.85**	**1.08**	**.46**	**90**	**100**
98/08/04	**5.75**	**24.40**	**25.87**	**8.93**	**0.87***	**19.57**	**0.00**	**2.84**	**0.00**	**.059**	**1.15**	**1.32**	**.47**	**92**	**102**

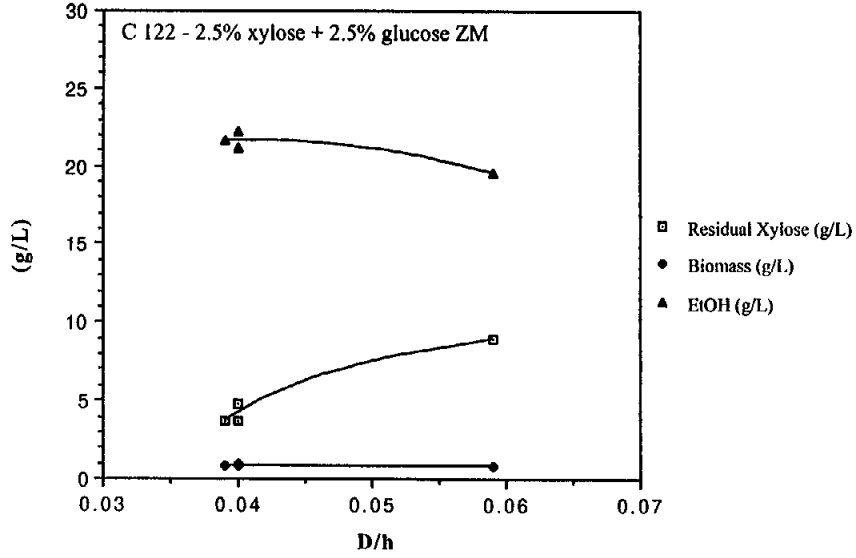
-terminated experiment

* biomass measured by filter

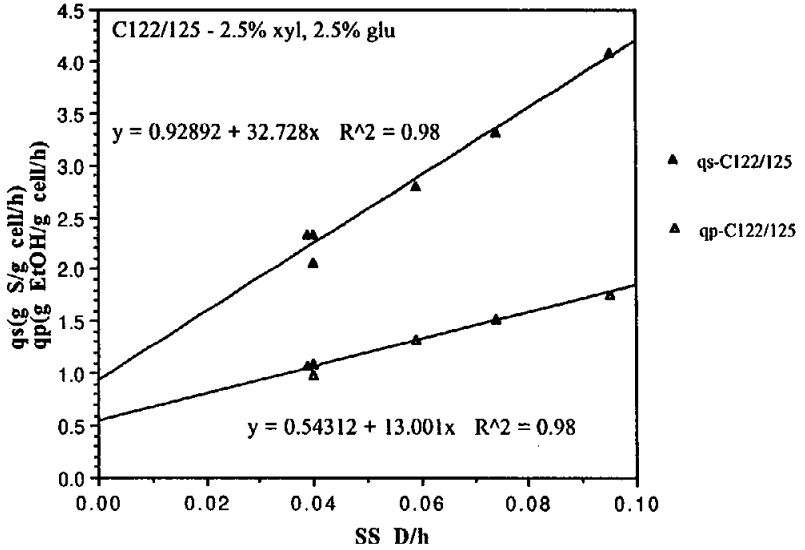
Growth of "adapted" ATCC 39676:pZB4L in ZM at pH 5.75 & 30°C



Growth of "adapted" ATCC 39676:pZB4L in 2.5% Xylose/Glucose ZM at pH 5.75 & 30°C



Continuous culture of "adapted" Zm ATCC 39676:pZB4L



C125- 98/09/15 - 98/10/02

OPERATIONAL PARAMETERS FOR CONTINUOUS FERMENTATIONS

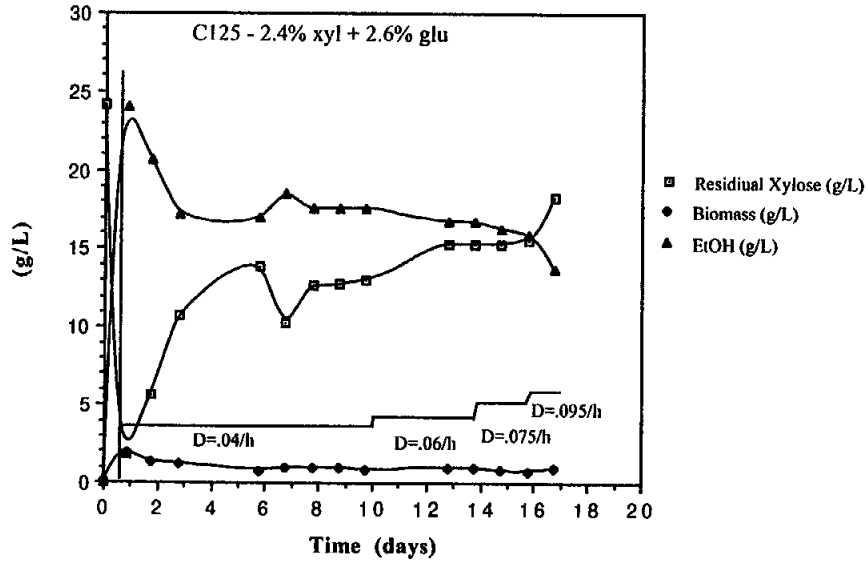
Sugar conversion by "adapted" *Zymomonas mobilis* ATCC 39676:pZB4L

		SUBSTRATE			[PRODUCTS]					PRODUCTIVITY			YIELD		
Date	pH	[Glu] g/L	[Xyl] g/L	Residual [Xyl] (g/L)	Biomass g/L	EtOH g/L	Xylitol g/L?	Lactate g/L	Acetate g/L	D hr^{-1}	Q_p g P/L/h	q_p g P/g cell/h	$Y_{p/s}$ g P/g Glu	Conversion Efffic (%)	% C Recovery
C125- Medium=RM(flow started 20h after inoculation)															
98/09/16	5.75	26.14	24.18	1.74	1.95*	23.98	0.00	0.00	0.00	.000	-	-	.49	96	101
Medium=1%cCSL + 1.8mM $MgSO_4$															
98/09/17	5.75	24.87	25.21	5.67	1.35	20.65	0.00	2.33	0.00	.040	0.83	0.61	.46	90	100
98/09/18	5.75	24.87	25.21	10.70G	1.20	17.27	0.00	3.55	0.00	.039	0.67	0.56	.48	94	107
98/09/21	**5.75**	**24.87**	**25.21**	**13.90**	**0.75**	**17.01**	**0.00**	**2.92**	**0.00**	**.039**	**0.66**	**0.88**	**.47**	**92**	**102**
-stopped flow for 22hours															
98/09/22	5.75	24.87	25.21	10.23	0.93	18.50	0.00	3.68	0.00	.000	-	-	.46	90	102
98/09/23	5.75	24.87	25.21	12.61	0.90	17.52	0.00	2.26	0.00	.039	0.68	0.76	.47	92	100
98/09/24	5.75	24.87	25.21	12.74	0.90	17.53	0.00	2.36	0.00	.039	0.68	0.80	.47	92	101
98/09/25	**5.75**	**24.87**	**25.21**	**13.03**	**0.87**	**17.57**	**0.00**	**2.54**	**0.00**	**.039**	**0.69**	**0.79**	**.47**	**92**	**102**
98/09/28	**5.75**	**24.87**	**25.21**	**15.35**	**0.93***	**16.72**	**0.00**	**3.30**	**0.00**	**.059**	**0.99**	**1.06**	**.48**	**94**	**105**
98/09/29	**5.75**	**24.87**	**25.21**	**15.29**	**0.93**	**16.72**	**0.00**	**3.08**	**0.00**	**.059**	**0.99**	**1.06**	**.48**	**94**	**104**
98/09/30	5.75	24.87	25.21	15.35	0.78	16.26	0.00	3.10	0.00	.076	1.24	1.58	.47	92	102
98/10/01	**5.75**	**24.87**	**25.21**	**15.58**	**0.77***	**15.92**	**0.00**	**2.56**	**0.00**	**.074**	**1.18**	**1.53**	**.46**	**90**	**100**
98/10/02	**5.75**	**24.87**	**25.21**	**18.28G**	**0.74***	**13.69**	**0.00**	**0.28**	**0.00**	**.095**	**1.30**	**1.76**	**.47**	**92**	**98**

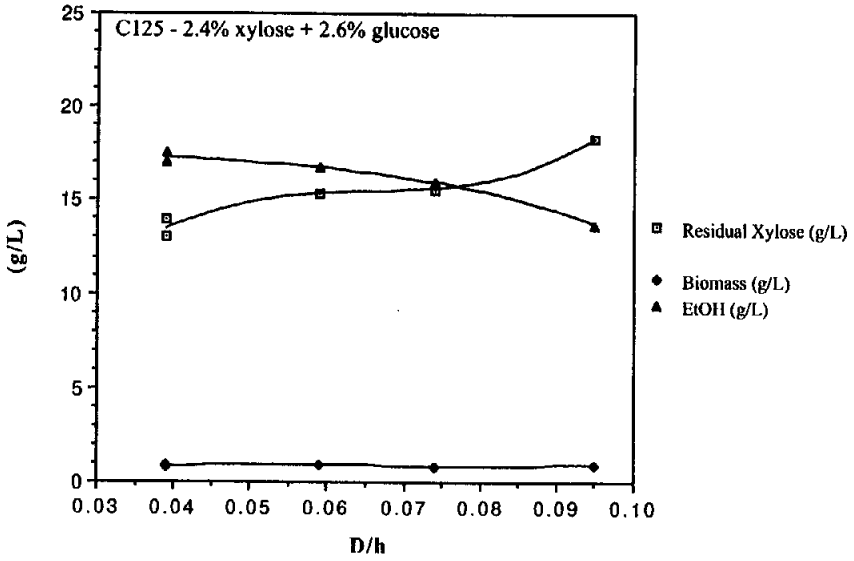
-terminated experiment

* biomass measured by filter

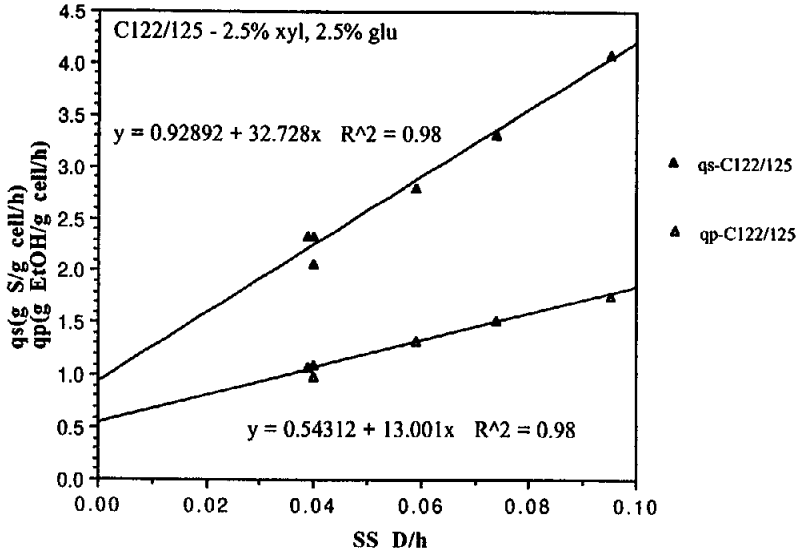
Growth of "adapted" ATCC 39676:pZB4L in 1%cCSL + 1.8mM Mg



Growth of "adapted" ATCC 39676:pZB4L in 1% cCSL + 1.8mM Mg



Continuous culture of "adapted" Zm ATCC 39676:pZB4L



C131 - 98/10/20-98/11/16

OPERATIONAL PARAMETERS FOR CONTINUOUS FERMENTATIONS

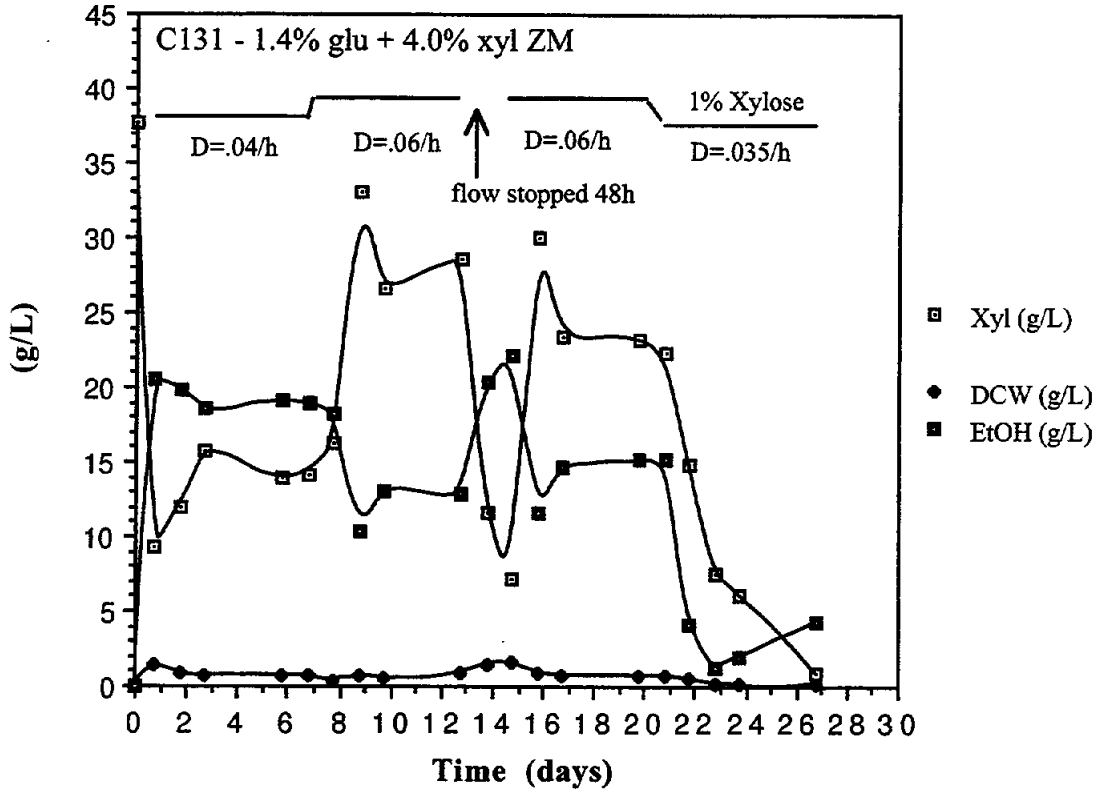
Sugar conversion by "xylose-adapted" *Zymomonas mobilis* ATCC 39676:pZB4L

		SUBSTRATE			[PRODUCTS]					PRODUCTIVITY			YIELD		
Date	pH	[Glu] g/L	[Xyl] g/L	Residual [Xyl] (g/L)	Biomass g/L	EtOH g/L	Xylitol g/L?	Lactate g/L	Acetate g/L	D hr^{-1}	Q_p g P/L/h	q_p g P/g cell/h	$Y_{p/s}$ g P/g Glu	Conversion Efffic (%)	% C Recovery
C131- Medium=ZM (flow started 18h after inoculation)															
98/10/21	5.75	12.93	37.63	9.36	1.47*	20.54	0.00	0.19	0.00	.000	-	-	.50	98	102
98/10/22	5.75	14.43	40.51	12.02	0.90	19.89	0.79	1.23	0.00	.038	0.76	0.84	.46	90	98
98/10/23	5.75	14.43	40.51	15.72	0.65	18.49	0.13	1.60	0.00	.037	0.68	1.05	.47	92	97
98/10/26	**5.75**	**14.43**	**40.51**	**13.92**	**0.69**	**19.03**	**0.00**	**1.92**	**0.00**	**.037**	**0.70**	**1.02**	**.46**	**90**	**99**
98/10/27	**5.75**	**14.43**	**40.51**	**14.15**	**0.66**	**18.88**	**0.00**	**2.16**	**0.00**	**.037**	**0.70**	**1.06**	**.46**	**90**	**98**
98/10/28	5.75	14.43	40.51	16.18	0.42	18.19	0.00	1.99	0.00	.062	1.13	2.69	.47	92	99
98/10/29	5.75	14.43	40.51	33.03	0.68	10.33	0.00	1.82	0.00	.061	0.63	0.93	.47	92	102
98/10/30	**5.75**	**14.43**	**40.51**	**26.62**	**0.53**	**13.05**	**0.00**	**0.32**	**0.00**	**.059**	**0.77**	**1.45**	**.48**	**94**	**98**
98/11/02	5.75	14.43	40.51	28.60	0.95	12.77	0.00	0.90	0.00	.060	0.77	0.81	.48	94	101
-stopped flow for 48h															
98/11/03	5.75	14.43	40.51	11.58	1.50	20.33	0.88	1.65	0.00	.000	-	-	.47	92	101
98/11/04	5.75	14.43	40.51	7.13	1.68	22.18	0.08	1.43	0.00	.000	-	-	.46	90	98
98/11/05	5.75	14.43	40.51	30.02	0.83	11.56	0.00	1.03	0.00	.059	0.68	0.82	.46	90	99
98/11/06	5.75	14.43	40.51	23.45	0.78	14.58	0.06	0.90	0.00	.060	0.87	1.12	.46	90	98
98/11/09	**5.75**	**14.43**	**40.51**	**23.16**	**0.78**	**15.09**	**0.00**	**1.70**	**0.00**	**.060**	**0.91**	**1.15**	**.47**	**92**	**102**
98/11/10	5.75	14.43	40.51	22.41	0.75	15.14	0.00	1.50	0.00	.036	0.55	0.73	.47	92	99
98/11/11	5.75	0.00	10.28	14.74	0.51	4.10	0.00	1.75	0.00	.035	-	-	-	-	-
98/11/12	5.75	0.00	10.28	7.51	0.20	1.30	0.00	0.28	0.00	.035	0.05	0.23	.47	92	103
98/11/13	5.75	0.00	10.28	6.06	0.10	2.02	0.00	0.13	0.00	.034	0.07	0.69	.48	94	100
98/11/16	**5.75**	**0.00**	**10.28**	**0.93**	**0.15**	**4.24**	**0.00**	**1.20**	**0.00**	**.035**	**0.15**	**0.99**	**.45**	**88**	**103**

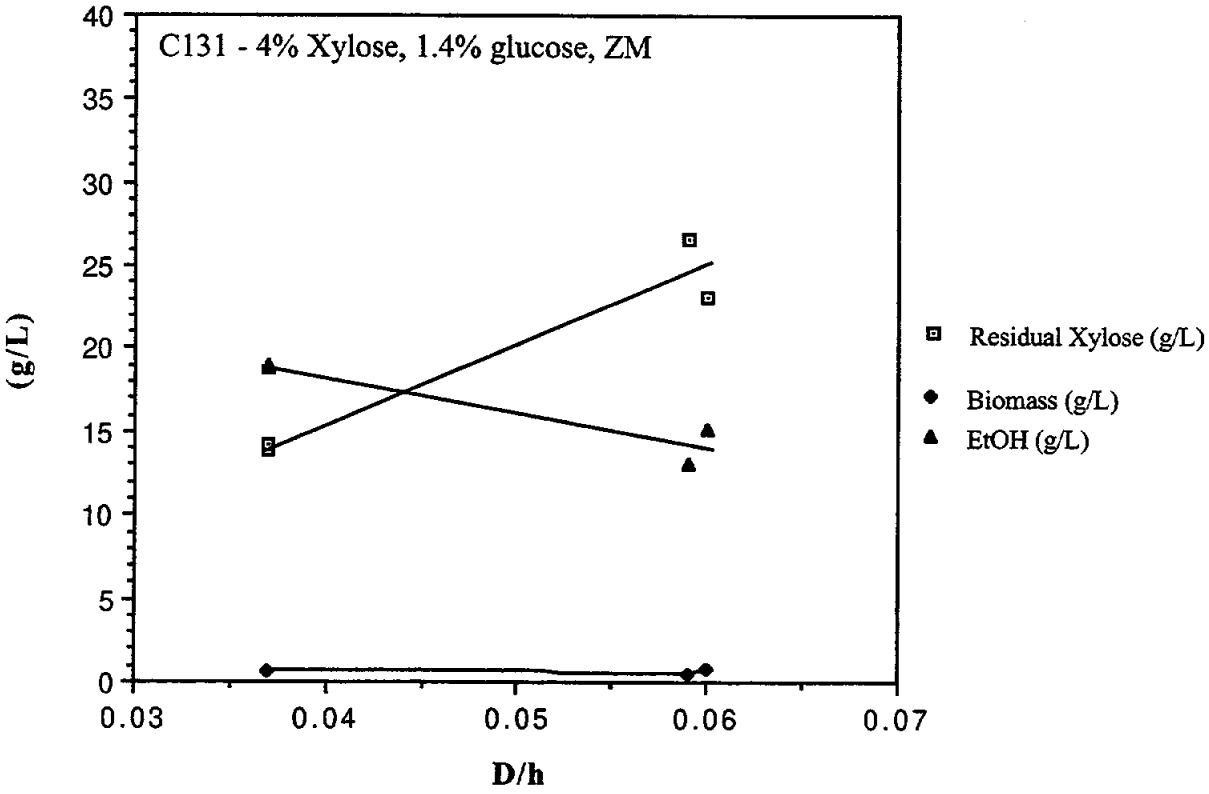
-terminated experiment due to contaminated reservoir

* biomass measured by filter

Growth of Zm "adapted" ATCC 39676:pZB4L in ZM at pH 5.75 & 30°C



Growth of "adapted" ATCC 39676:pZB4L at pH 5.75 & 30°C



APPENDIX F

Summaries of chemostat experiments for Task 4

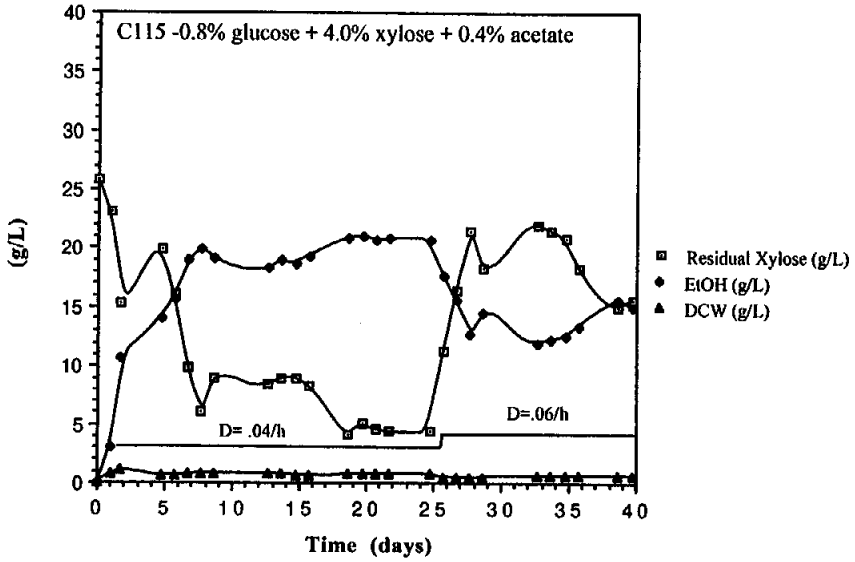
C115- 98/05/06-98/06/16

OPERATIONAL PARAMETERS FOR CONTINUOUS FERMENTATIONS

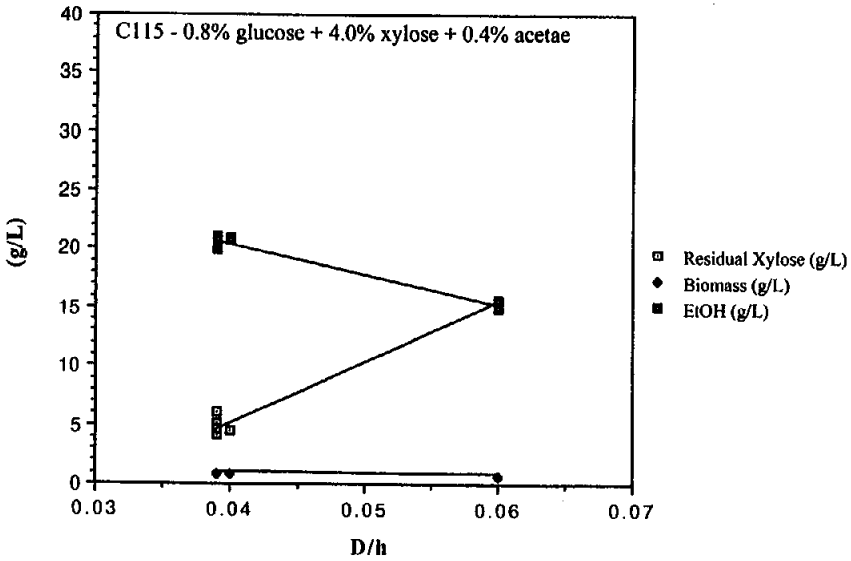
Sugar conversion by "adapted" *Zymomonas mobilis* ATCC 39676:pZB4L

		SUBSTRATE			[PRODUCTS]					PRODUCTIVITY			YIELD		
Date	pH	[Glu] g/L	[Xyl] g/L	Residual [Xyl] (g/L)	Biomass g/L	EtOH g/L	Xylitol g/L?	Lactate g/L	Acetate g/L	D hr^{-1}	Q_p g P/L/h	q_p g P/g cell/h	$Y_{p/s}$ g P/g Glu	Conversion Efffic (%)	% C Recovery
C115- Medium=1%cCSL + tap H_2O + 0.4% acetate (flow started 40h after inoculation)															
98/05/07	5.75	21.27	25.75	23.04	0.75	3.07	0.00	0.06	0.00	.000	-	-	.49	96	102
98/05/08	5.75	21.27	25.75	15.28	1.07	10.61	0.00	0.13	0.00	.000	-	-	.50	98	102
98/05/11	5.75	7.89	40.72	19.97	0.58	14.10	0.00	1.87	4.00	.037	0.52	0.90	.49	96	103
98/05/12	5.75	7.89	40.72	16.16	0.58	15.65	0.00	1.69	4.00	.039	0.61	1.05	.48	94	101
98/05/13	**5.75**	**7.89**	**40.72**	**9.88**	**0.86**	**18.93**	**0.00**	**1.45**	**4.00**	**.039**	**0.74**	**0.86**	**.49**	**96**	**102**
98/05/14	**5.75**	**7.89**	**40.72**	**6.05**	**0.81***	**19.92**	**0.00**	**1.93**	**4.00**	**.039**	**0.78**	**0.96**	**.47**	**92**	**99**
98/05/15	5.75	7.89	40.72	8.94	0.81	19.14	0.00	1.08	4.00	.039	0.75	0.92	.48	94	100
98/05/19	5.75	7.99	40.53	8.40	0.81	18.30	0.00	2.04	4.00	.038	0.70	0.86	.46	90	97
98/05/20	5.75	7.99	40.53	8.98	0.76	18.98	0.00	1.90	4.00	.037	0.70	0.92	.48	94	101
98/05/21	5.75	7.99	40.53	8.99	0.63	18.68	0.00	1.23	4.00	.039	0.73	1.16	.47	92	98
98/05/22	5.75	8.40	40.12	8.28	0.63	19.29	0.00	1.26	4.00	.039	0.75	1.19	.48	94	99
98/05/25	**5.75**	**8.40**	**40.12**	**4.09**	**0.72***	**20.88**	**0.00**	**1.79**	**4.00**	**.039**	**0.81**	**1.13**	**.47**	**92**	**98**
98/05/26	**5.75**	**8.40**	**40.12**	**5.02**	**0.72**	**21.07**	**0.00**	**1.08**	**4.00**	**.039**	**0.82**	**1.14**	**.48**	**94**	**99**
98/05/27	**5.75**	**8.40**	**40.12**	**4.63**	**0.72***	**20.76**	**0.00**	**1.36**	**4.00**	**.039**	**0.81**	**1.12**	**.48**	**94**	**98**
98/05/28	**5.75**	**8.06**	**40.23**	**4.41**	**0.72**	**20.80**	**0.00**	**1.51**	**4.00**	**.040**	**0.83**	**1.16**	**.47**	**92**	**98**
98/06/01	**5.75**	**8.06**	**40.23**	**4.41**	**0.72**	**20.76**	**0.00**	**1.68**	**4.00**	**.040**	**0.83**	**1.15**	**.47**	**92**	**99**
98/06/02	5.75	8.06	40.23	11.36	0.49	17.75	0.00	1.18	4.00	.060	1.07	2.17	.48	94	99
98/06/03	5.75	8.07	40.15	16.41	0.52	15.58	0.00	1.30	4.00	.060	0.93	1.80	.49	96	101
98/06/04	**5.75**	**8.07**	**40.15**	**21.55**	**0.52**	**12.80**	**0.00**	**1.30**	**4.00**	**.060**	**0.77**	**1.48**	**.48**	**94**	**101**
98/06/05	**5.75**	**8.07**	**40.15**	**18.28**	**0.55**	**14.45**	**0.00**	**1.28**	**4.00**	**.060**	**0.87**	**1.58**	**.48**	**94**	**101**
98/06/09	5.75	8.05	39.63	22.04	0.58	12.02	0.00	1.23	4.00	.060	0.72	1.24	.47	92	100
98/06/10	5.75	8.05	39.63	21.49	0.58	12.32	0.00	1.20	4.00	.060	0.74	1.27	.47	92	100
98/06/11	5.75	8.05	39.63	20.94	0.63	12.56	0.00	1.43	4.00	.060	0.75	1.20	.47	92	100
98/06/12	**5.75**	**8.05**	**39.63**	**18.31**	**0.69**	**13.42**	**0.00**	**1.47**	**4.00**	**.060**	**0.81**	**1.17**	**.46**	**90**	**98**
98/06/15	**5.75**	**8.05**	**39.63**	**14.94**	**0.58**	**15.62**	**0.00**	**1.41**	**4.00**	**.060**	**0.94**	**1.62**	**.48**	**94**	**100**
98/06/16	**5.75**	**8.05**	**39.63**	**15.59**	**0.66**	**14.98**	**0.00**	**1.68**	**4.00**	**.060**	**0.90**	**1.36**	**.47**	**92**	**100**

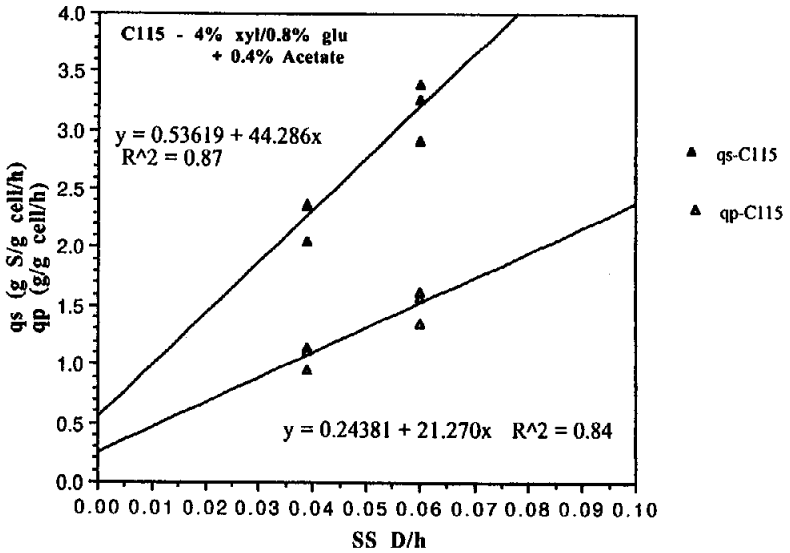
Growth of "adapted" ATCC 396765:pZB4L in 1%cCSL + tap water at pH 5.75 & 30°C



Growth of "adapted" ATCC 39676:pZB4L in 1%cCSL + tap water at pH 5.75 & 30°C



Continuous culture of "adapted" Zm ATCC 39676:pZB4L



C117- 98/06/17-98/07/02

OPERATIONAL PARAMETERS FOR CONTINUOUS FERMENTATIONS

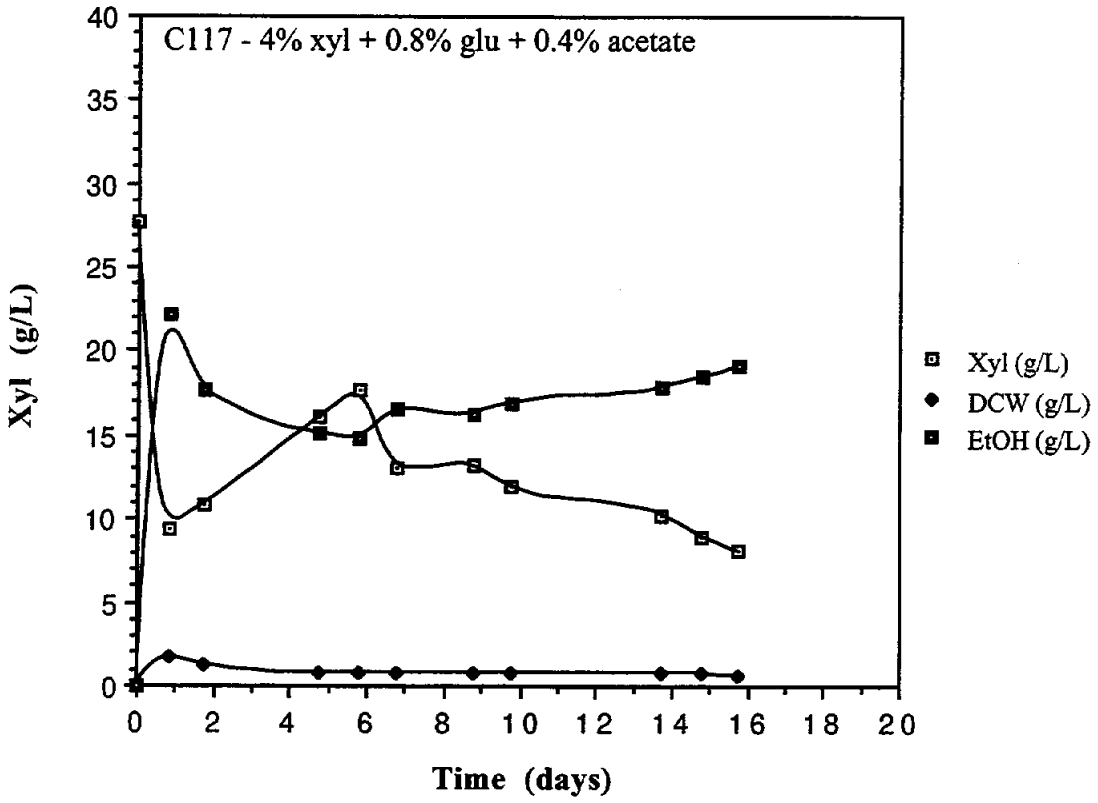
Sugar conversion by "adapted" *Zymomonas mobilis* ATCC 39676:pZB4L

		SUBSTRATE			[PRODUCTS]					PRODUCTIVITY			YIELD		
Date	pH	[Glu] g/L	[Xyl] g/L	Residual [Xyl] (g/L)	Biomass g/L	EtOH g/L	Xylitol g/L?	Lactate g/L	Acetate g/L	D hr^{-1}	Q_p g P/L/h	q_p g P/g cell/h	$Y_{p/s}$ g P/g Glu	Conversion Efffic (%)	% C Recovery
C117- Medium=1%cCSL +Zymo salts + tap H_2O + 0.4% acetate (flow started 20h after inoculation)															
98/06/18	5.75	25.95	27.79	9.47	1.81*	22.12	0.00	0.00	0.00	.000	-	-	.49	96	102
98/06/19	5.75	7.61	40.36	10.85	1.24	17.66	0.00	1.47	3.8	.040	0.71	0.57	.47	92	101
98/06/22	**5.75**	**7.61**	**40.36**	**16.10**	**0.75***	**15.13**	**0.00**	**1.12**	**3.8**	**.040**	**0.61**	**0.81**	**.47**	**92**	**99**
98/06/23	**5.75**	**7.61**	**40.36**	**17.76**	**0.75**	**14.83**	**0.00**	**1.18**	**4.0**	**.040**	**0.59**	**0.79**	**.49**	**96**	**102**
98/06/24	**5.75**	**7.61**	**40.36**	**13.03**	**0.75**	**16.51**	**0.00**	**1.28**	**4.0**	**.040**	**0.66**	**0.88**	**.47**	**92**	**99**
98/06/25	**5.75**	**7.61**	**40.36**	**13.28**	**0.78**	**16.29**	**0.00**	**1.12**	**4.0**	**.040**	**0.65**	**0.84**	**.47**	**92**	**98**
Medium=1%cCSL +Zymo salts + D H_2O + 0.4% acetate															
98/06/26	5.75	7.79	39.66	11.95	0.75*	16.92	0.00	1.39	4.0	.040	0.68	0.90	.48	94	100
98/06/30	**5.75**	**7.79**	**39.66**	**10.25**	**0.75**	**17.85**	**0.00**	**1.46**	**4.0**	**.040**	**0.71**	**0.95**	**.48**	**94**	**100**
98/07/01	**5.75**	**7.79**	**39.66**	**9.00**	**0.72**	**18.45**	**0.00**	**1.52**	**4.0**	**.040**	**0.74**	**1.03**	**.48**	**94**	**100**
98/07/02	**5.75**	**7.79**	**39.66**	**8.06**	**0.66**	**19.05**	**0.00**	**1.78**	**4.0**	**.040**	**0.76**	**1.15**	**.48**	**94**	**101**

-terminated experiment due to lack of xylose

* biomass measured by filter

Growth of "adapted" ATCC 39676:pZB4L in 1% cCSL + Z salts at pH 5.75 &30°C



C124- 98/09/16 - 98/10/05

OPERATIONAL PARAMETERS FOR CONTINUOUS FERMENTATIONS

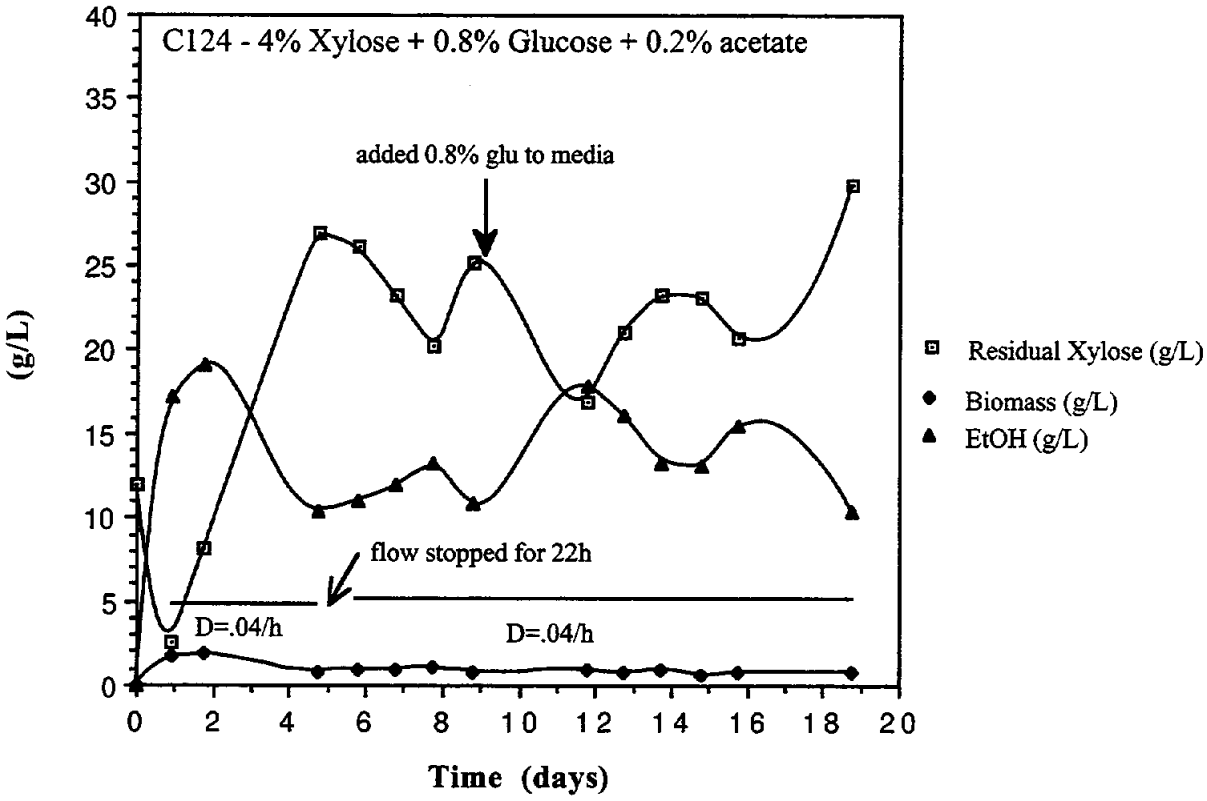
Sugar conversion by "adapted" *Zymomonas mobilis* ATCC 39676:pZB4L

		SUBSTRATE			[PRODUCTS]					PRODUCTIVITY			YIELD		
Date	pH	[Glu]	[Xyl]	Residual	Biomass	EtOH	Xylitol	Lactate	Acetate	D	Q_p	q_p	$Y_{p/s}$	Conversion	% C
		g/L	g/L	[Xyl] (g/L)	g/L	g/L	g/L?	g/L	g/L	hr^{-1}	g P/L/h	g P/g cell/h	g P/g Glu	Efffic (%)	Recovery
C124- Medium=RM(flow started 22h after inoculation)															
98/09/17	5.75	26.53	12.00	2.51	1.68*	17.24	0.00	0.02	0.00	.000	-	-	.48	94	99
Medium=1%cCSL + 1.8mM $MgSO_4$ + 0.2% acetate															
98/09/18	5.75	8.36	40.22	8.11	1.86	19.20	0.00	3.13	2.0*	.039	0.75	0.40	.47	92	105
98/09/21	**5.75**	**8.36**	**40.22**	**26.99**	**0.84**	**10.43**	**0.00**	**1.89**	**2.0***	**.041**	**0.43**	**0.51**	**.48**	**94**	**104**
-stopped flow for 22hours															
98/09/22	5.75	8.36	40.22	26.17	0.93	10.93	0.00	2.43	2.0*	.000	-	-	.49	96	105
98/09/23	5.75	8.36	40.22	23.23	1.02	11.92	0.00	1.85	2.0*	.040	0.48	0.47	.47	92	102
98/09/24	5.75	8.36	40.22	20.17	1.08	13.17	0.00	2.38	2.0*	.039	0.51	0.48	.46	90	102
98/09/25	**5.75**	**8.36**	**40.22**	**25.19**	**0.84**	**10.84**	**0.00**	**1.69**	**2.0***	**.039**	**0.42**	**0.50**	**.46**	**90**	**101**
98/09/28	5.75	15.55	39.15	16.97	0.92*	17.89	0.00	2.91	2.0*	.039	0.70	0.76	.47	92	103
98/09/29	**5.75**	**15.55**	**39.15**	**21.06**	**0.75***	**16.07**	**0.00**	**1.75**	**2.0***	**.040**	**0.64**	**0.86**	**.48**	**94**	**101**
98/09/30	5.75	15.55	39.15	23.31G	0.92	13.28	0.00	1.43	2.0*	.039	0.52	0.56	.46	90	100
98/10/01	5.75	15.55	39.15	23.08G	0.70	13.03	0.00	2.06	2.0*	.041	0.52	0.74	.47	92	101
98/10/02	5.75	15.55	39.15	20.78G	0.73	15.50	0.00	2.05	2.0*	.041	0.64	0.87	.47	92	100
98/10/05	5.75	15.55	39.15	29.73	0.77	10.37	0.00	6.41	2.0*	.040	0.41	0.54	.42	82	105

-terminated experiment due to contaminated reservoir

* biomass measured by filter

Growth of "adapted" ATCC 39676:pZB4L in 1% cCSL + 1.8mM Mg + 0.2% acetate at pH 5.75 & 30°C



C114- 98/04/16-98/05/19

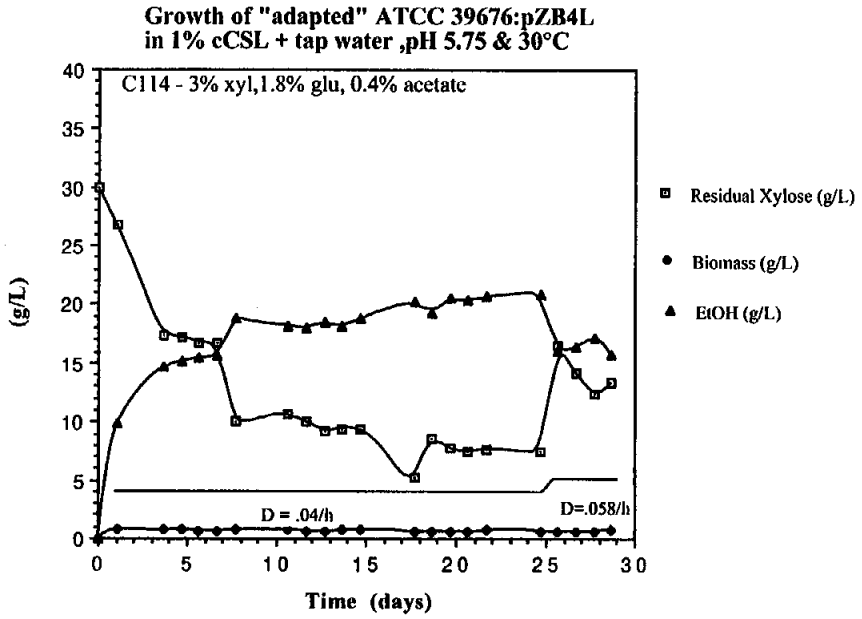
OPERATIONAL PARAMETERS FOR CONTINUOUS FERMENTATIONS

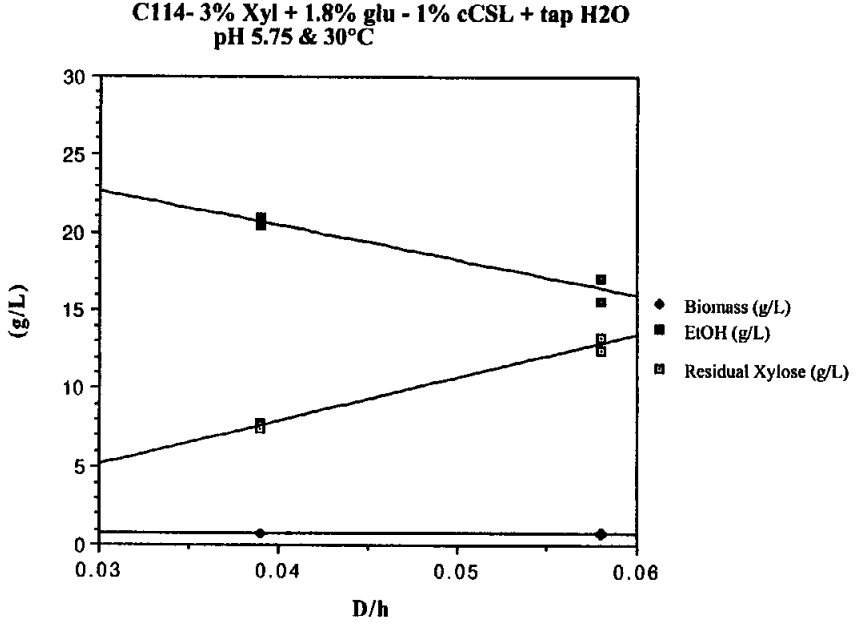
Sugar conversion by "adapted" *Zymomonas mobilis* ATCC 39676:pZB4L

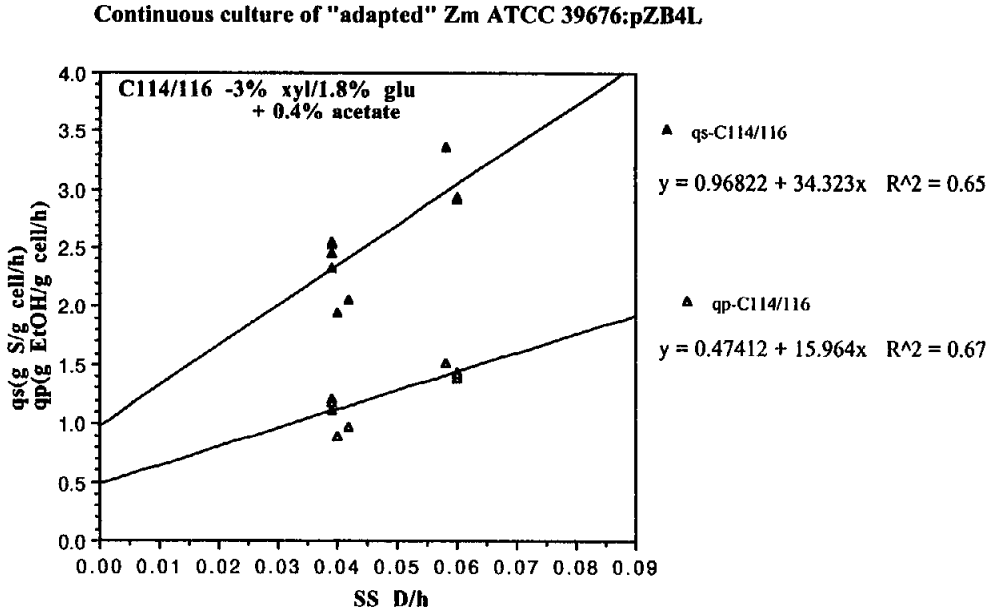
		SUBSTRATE			[PRODUCTS]					PRODUCTIVITY			YIELD		
Date	pH	[Glu] g/L	[Xyl] g/L	Residual [Xyl] (g/L)	Biomass g/L	EtOH g/L	Xylitol g/L?	Lactate g/L	Acetate g/L	D hr^{-1}	Q_p g P/L/h	q_p g P/g cell/h	$Y_{p/s}$ g P/g Glu	Conversion Efffic (%)	% C Recovery
C114- Medium=1%cCSL + tap H_2O + 0.4% acetate (flow started 24h after inoculation)															
98/04/17	5.75	18.01	30.02	26.85	0.81*	9.93	0.00	1.98	3.90	.000	-	-	.47	92	102
98/04/20	**5.75**	**18.01**	**30.02**	**17.39**	**0.72**	**14.64**	**0.00**	**1.65**	**3.90**	**.040**	**0.59**	**0.81**	**.48**	**94**	**101**
98/04/21	**5.75**	**18.16**	**30.01**	**17.20**	**0.81**	**15.14**	**0.00**	**2.15**	**4.00**	**.040**	**0.61**	**0.75**	**.49**	**96**	**104**
98/04/22	**5.75**	**18.16**	**30.01**	**16.80**	**0.69**	**15.38**	**0.00**	**1.71**	**4.00**	**.040**	**0.62**	**0.89**	**.49**	**96**	**103**
98/04/23	**5.75**	**18.16**	**30.01**	**16.75**	**0.66**	**15.55**	**0.00**	**1.80**	**4.00**	**.040**	**0.62**	**0.94**	**.49**	**96**	**103**
98/04/24	**5.75**	**18.16**	**30.01**	**9.97**	**0.75***	**18.73**	**0.00**	**2.36**	**4.00**	**.039**	**0.73**	**0.97**	**.49**	**96**	**103**
98/04/27	**5.75**	**18.16**	**30.01**	**10.66**	**0.84**	**18.18**	**0.00**	**2.39**	**4.00**	**.038**	**0.69**	**0.82**	**.48**	**94**	**103**
98/04/28	**5.75**	**18.14**	**30.22**	**9.98**	**0.65**	**18.04**	**0.00**	**2.61**	**3.90**	**.038**	**0.69**	**1.05**	**.47**	**92**	**101**
98/04/29	**5.75**	**18.14**	**30.22**	**9.28**	**0.65**	**18.54**	**0.00**	**2.61**	**3.90**	**.038**	**0.70**	**1.08**	**.47**	**92**	**101**
98/04/30	**5.75**	**18.14**	**30.22**	**9.48**	**0.72***	**18.14**	**0.00**	**2.97**	**3.90**	**.039**	**0.71**	**0.98**	**.47**	**92**	**101**
98/05/01	**5.75**	**18.14**	**30.22**	**9.40**	**0.78**	**18.76**	**0.00**	**2.77**	**3.90**	**.039**	**0.73**	**0.94**	**.48**	**94**	**103**
98/05/04	**5.75**	**18.14**	**30.22**	**5.18**	**0.69**	**20.16**	**0.00**	**2.08**	**3.90**	**.039**	**0.79**	**1.14**	**.47**	**92**	**98**
98/05/05	**5.75**	**19.52**	**31.19**	**8.64**	**0.66**	**19.36**	**0.00**	**2.23**	**3.90**	**.039**	**0.76**	**1.14**	**.46**	**90**	**98**
98/05/06	**5.75**	**19.52**	**31.19**	**7.73**	**0.66***	**20.49**	**0.00**	**2.33**	**3.90**	**.039**	**0.80**	**1.21**	**.48**	**94**	**100**
98/05/07	**5.75**	**19.52**	**31.19**	**7.51**	**0.66**	**20.44**	**0.00**	**2.20**	**3.90**	**.039**	**0.80**	**1.21**	**.47**	**92**	**100**
98/05/08	**5.75**	**19.52**	**31.19**	**7.64**	**0.72***	**20.65**	**0.00**	**2.52**	**3.90**	**.039**	**0.80**	**1.12**	**.48**	**94**	**101**
98/05/11	**5.75**	**19.52**	**31.19**	**7.45**	**0.69**	**20.90**	**0.83**	**1.54**	**4.00**	**.039**	**0.82**	**1.18**	**.48**	**94**	**102**
98/05/12	5.75	19.52	31.19	16.51	0.63	16.12	0.00	1.32	4.00	.058	0.93	1.48	.47	92	99
98/05/13	5.75	19.52	31.19	14.14	0.63	16.36	0.00	1.70	4.00	.058	0.95	1.51	.45	88	100
98/05/14	**5.75**	**19.52**	**31.19**	**12.38**	**0.66***	**17.15**	**0.42**	**1.54**	**4.00**	**.058**	**0.99**	**1.51**	**.45**	**88**	**101**
98/05/15	**5.75**	**17.62**	**29.19**	**13.31**	**0.68***	**15.70**	**0.00**	**1.71**	**4.00**	**.058**	**0.91**	**1.34**	**.47**	**92**	**100**

98/05/19 - temperature dropped to 15° C & reservoir is contaminated --terminated experiment

* Biomass measured by filter method;
only lines in **bold** are at steady state


Growth of "adapted" ATCC 39676:pZB4L
in 1% cCSL + tap water ,pH 5.75 & 30°C
C114 - 3% xyl,1.8% glu, 0.4% acetate
(g/L)
Time (days)
D = .04/h
D=.058/h
Residual Xylose (g/L)
Biomass (g/L)
EtOH (g/L)


C114- 3% Xyl + 1.8% glu - 1% cCSL + tap H2O
pH 5.75 & 30°C
(g/L)
D/h
Biomass (g/L)
EtOH (g/L)
Residual Xylose (g/L)


Continuous culture of "adapted" Zm ATCC 39676:pZB4L
C114/116 -3% xyl/1.8% glu
+ 0.4% acetate
qs(g S/g cell/h)
qp(g EtOH/g cell/h)
SS D/h
qs-C114/116
y = 0.96822 + 34.323x R^2 = 0.65
qp-C114/116
y = 0.47412 + 15.964x R^2 = 0.67

C116- 98/05/21-98/06/18

OPERATIONAL PARAMETERS FOR CONTINUOUS FERMENTATIONS

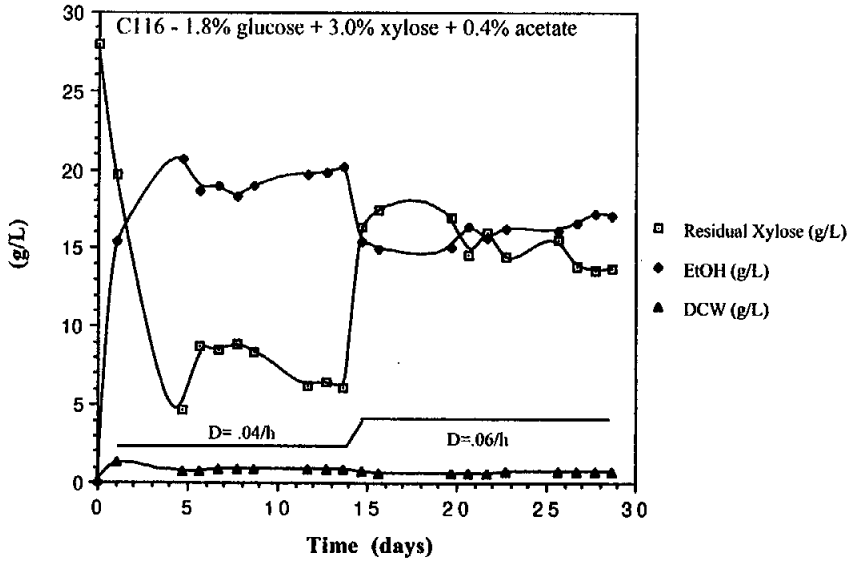
Sugar conversion by "adapted" *Zymomonas mobilis* ATCC 39676:pZB4L

		SUBSTRATE			[PRODUCTS]					PRODUCTIVITY			YIELD		
Date	pH	[Glu] g/L	[Xyl] g/L	Residual [Xyl] (g/L)	Biomass g/L	EtOH g/L	Xylitol g/L?	Lactate g/L	Acetate g/L	D hr^{-1}	Q_p g P/L/h	q_p g P/g cell/h	$Y_{p/s}$ g P/g Glu	Conversion Efffic (%)	% C Recovery
C116- Medium=1%cCSL + tap H_2O + 0.4% acetate (flow started 24h after inoculation)															
98/05/22	5.75	22.75	27.91	19.68	1.30	15.47	0.00	0.26	0.00	.000	-	-	.50	98	102
98/05/25	5.75	18.08	30.12	4.70	0.72	20.69	0.00	1.23	4.0	.039	0.81	1.12	.48	94	98
98/05/26	5.75	18.08	30.12	8.69	0.72	18.70	0.00	1.99	4.0	.039	0.73	1.01	.47	92	100
98/05/27	5.75	18.08	30.12	8.49	0.81	18.96	0.00	1.99	4.0	.040	0.76	0.94	.47	92	101
98/05/28	**5.75**	**18.08**	**30.12**	**8.87**	**0.81**	**18.24**	**0.00**	**2.18**	**4.0**	**.040**	**0.73**	**0.90**	**.46**	**90**	**99**
98/05/29	**5.75**	**18.08**	**30.12**	**8.41**	**0.81**	**18.96**	**0.00**	**1.88**	**4.0**	**.042**	**0.80**	**0.98**	**.48**	**94**	**100**
98/06/01	**5.75**	**18.08**	**30.12**	**6.24**	**0.84**	**19.78**	**0.00**	**2.05**	**4.0**	**.040**	**0.79**	**0.94**	**.47**	**92**	**100**
98/06/02	**5.75**	**18.08**	**30.12**	**6.42**	**0.84**	**19.80**	**0.00**	**1.76**	**4.0**	**.040**	**0.79**	**0.94**	**.47**	**92**	**99**
98/06/03	**5.75**	**18.08**	**30.12**	**6.06**	**0.84**	**20.20**	**0.00**	**1.50**	**4.0**	**.040**	**0.81**	**0.96**	**.48**	**94**	**100**
98/06/04	5.75	18.23	30.21	16.42	0.69	15.36	0.00	1.35	4.0	.060	0.92	1.34	.48	94	100
98/06/05	5.75	18.23	30.21	17.46	0.60	14.98	0.00	1.36	4.0	.060	0.90	1.50	.48	94	101
98/06/09	5.75	18.23	30.21	17.03	0.60	15.11	0.00	1.36	4.0	.060	0.91	1.51	.48	94	100
98/06/10	5.75	18.31	30.54	14.63	0.63	16.43	0.00	1.31	4.0	.060	0.99	1.56	.48	94	100
98/06/11	5.75	18.31	30.54	15.97	0.63	15.67	0.00	2.04	4.0	.060	0.94	1.49	.48	94	101
98/06/12	5.75	18.31	30.54	14.42	0.66	16.21	0.00	1.53	4.0	.060	0.97	1.47	.47	92	99
98/06/15	5.75	18.31	30.54	15.50	0.72	16.17	0.00	1.68	4.0	.060	0.97	1.35	.48	94	102
98/06/16	**5.75**	**18.31**	**30.54**	**13.88**	**0.72**	**16.60**	**0.00**	**1.94**	**4.0**	**.060**	**1.00**	**1.38**	**.47**	**92**	**101**
98/06/17	**5.75**	**18.31**	**30.54**	**13.63**	**0.72**	**17.19**	**0.00**	**1.85**	**4.0**	**.060**	**1.03**	**1.43**	**.49**	**96**	**100**
98/06/18	**5.75**	**18.31**	**30.54**	**13.72**	**0.72**	**17.09**	**0.00**	**1.22**	**4.0**	**.060**	**1.03**	**1.42**	**.49**	**96**	**101**

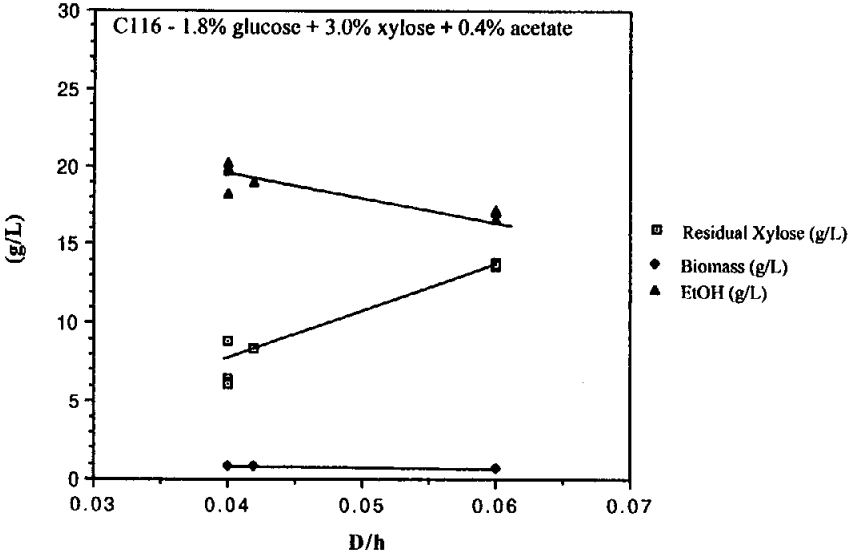
-terminated experiment

* Biomass measured by filter method;
only lines in **bold** are at steady state

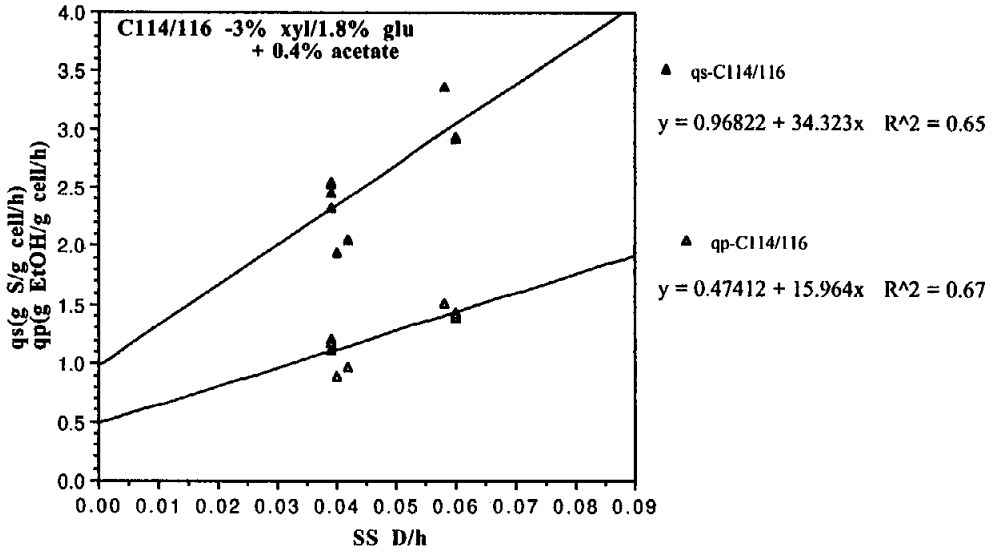
Growth of "adapted" ATCC 396765:pZB4L in 1%cCSL + tap water at pH 5.75 & 30°C



Growth of "adapted" ATCC 39676:pZB4L in 1%cCSL + tap water at pH 5.75 & 30°C



Continuous culture of "adapted" Zm ATCC 39676:pZB4L



C118- 98/06/18-98/07/02

OPERATIONAL PARAMETERS FOR CONTINUOUS FERMENTATIONS

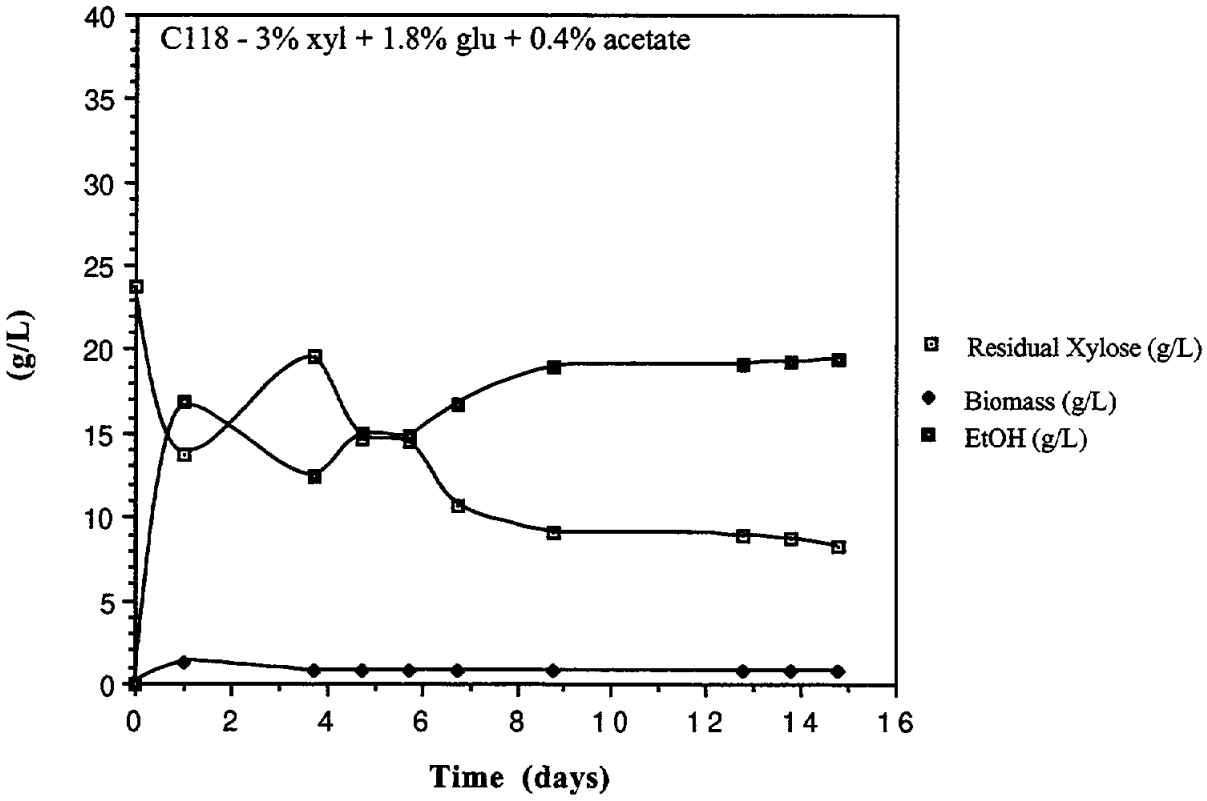
Sugar conversion by "adapted" *Zymomonas mobilis* ATCC 39676:pZB4L

		SUBSTRATE			[PRODUCTS]					PRODUCTIVITY			YIELD		
Date	pH	[Glu] g/L	[Xyl] g/L	Residual [Xyl] (g/L)	Biomass g/L	EtOH g/L	Xylitol g/L?	Lactate g/L	Acetate g/L	D hr^{-1}	Q_p g P/L/h	q_p g P/g cell/h	$Y_{p/s}$ g P/g Glu	Conversion Efffic (%)	% C Recovery
C118- Medium=1%cCSL +Zymo salts + tap H_2O + 0.4% acetate (flow started 24h after inoculation)															
98/06/19	5.75	25.21	23.71	13.71	1.32	16.89	0.00	0.12	0.0	.000	-	-	.48	94	99
98/06/22	5.75	16.77	29.27	19.61	0.82	12.42	0.00	1.43	4.0	.040	0.50	0.61	.47	92	101
98/06/23	**5.75**	**16.77**	**29.27**	**14.67**	**0.78**	**14.98**	**0.00**	**1.92**	**4.0**	**.040**	**0.60**	**0.77**	**.48**	**94**	**102**
98/06/24	**5.75**	**16.77**	**29.27**	**14.58**	**0.78**	**14.86**	**0.00**	**1.88**	**4.0**	**.040**	**0.59**	**0.76**	**.47**	**92**	**101**
98/06/25	**5.75**	**16.77**	**29.27**	**10.70**	**0.78**	**16.79**	**0.00**	**1.74**	**4.0**	**.040**	**0.65**	**0.84**	**.48**	**94**	**100**
Medium=1%cCSL +Zymo salts + D H_2O + 0.4% acetate															
98/06/26	5.75	18.20	30.03	9.14	0.78	18.99	0.00	1.83	4.0	.040	0.76	0.97	.49	96	102
98/06/30	**5.75**	**18.20**	**30.03**	**8.94**	**0.78**	**19.18**	**0.00**	**1.38**	**4.0**	**.040**	**0.77**	**0.98**	**.49**	**96**	**101**
98/07/01	**5.75**	**18.20**	**30.03**	**8.78**	**0.78**	**19.25**	**0.00**	**1.72**	**4.0**	**.040**	**0.77**	**0.99**	**.49**	**96**	**102**
98/07/02	**5.75**	**18.20**	**30.03**	**8.32**	**0.81**	**19.42**	**0.00**	**1.76**	**4.0**	**.040**	**0.78**	**0.96**	**.49**	**96**	**102**

-terminated experiment due to lack of xylose

* biomass measured by filter

Growth of "adapted" ATCC 39676:pZB4L in 1% cCSL + Z salts at pH 5.75 &30°C



C119- 98/07/14 - 98/08/04

OPERATIONAL PARAMETERS FOR CONTINUOUS FERMENTATIONS

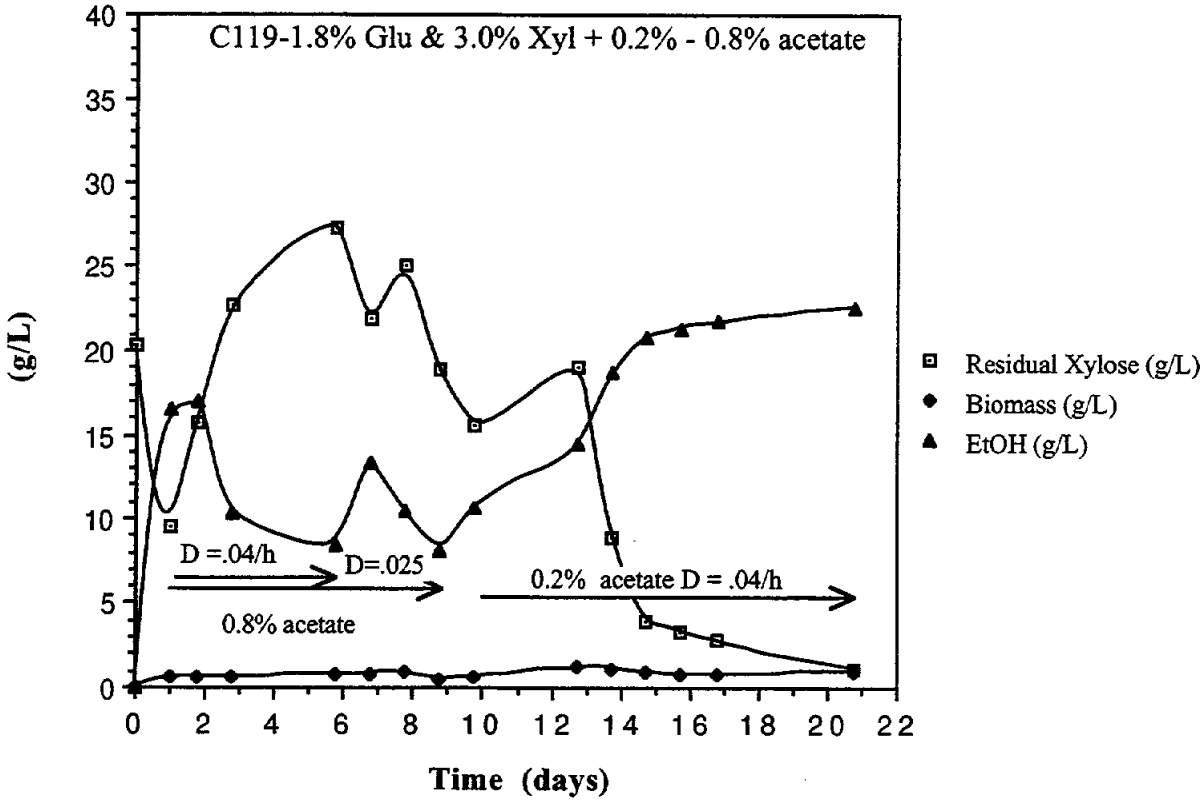
Sugar conversion by "adapted" *Zymomonas mobilis* ATCC 39676:pZB4L

		SUBSTRATE			[PRODUCTS]					PRODUCTIVITY			YIELD		
Date	pH	[Glu]	[Xyl]	Residual	Biomass	EtOH	Xylitol	Lactate	Acetate	D	Q_p	q_p	$Y_{p/s}$	Conversion	% C
		g/L	g/L	[Xyl] (g/L)	g/L	g/L	g/L?	g/L	g/L	hr^{-1}	g P/L/h	g P/g cell/h	g P/g Glu	Efffic (%)	Recovery
C119- Medium=ZM(flow started 24h after inoculation)															
98/07/15	5.75	22.48	20.27	9.57	0.60	16.45	0.00	0.00	0.0	.000	-	-	.50	98	99
Medium=2%cCSL(NACAN) +Zymo salts + 0.8% acetate															
98/07/16	5.75	18.21	32.12	15.68	0.66	16.99	0.00	0.80	4.89	.040	0.68	1.03	.49	96	99
98/07/17	5.75	18.21	32.12	22.64G	0.66	10.29	0.00	2.62	8.10	.040	0.41	0.62	.45	88	101
98/07/20	**5.75**	**18.21**	**32.12**	**27.38G**	**0.81***	**8.43**	**0.00**	**2.04**	**8.10**	**.040**	**0.34**	**0.42**	**.47**	**92**	**103**
98/07/21	5.75	18.21	32.12	21.92	0.87	13.34	0.00	2.54	8.10	.025	0.33	0.38	.47	92	103
98/07/22	5.75	18.21	32.12	25.15G	0.93	10.47	0.00	2.32	8.10	.025	0.26	0.28	.48	94	104
Medium=1%cCSL(NACAN) +Zymo salts + 0.2% acetate															
98/07/23	5.75	18.39	30.29	18.89G	0.50	8.09	0.00	0.76	6.60	.039	0.32	0.63	.46	90	99
98/07/24	5.75	18.39	30.29	15.60G	0.69	10.65	0.00	0.86	2.30	.039	0.42	0.60	.46	90	98
98/07/27	5.75	18.39	30.29	19.00	1.20	14.38	0.22	1.83	2.20	.040	0.58	0.48	.48	94	104
98/07/28	5.75	18.39	30.29	8.85	1.17	18.76	0.32	2.04	2.00	.041	0.77	0.66	.47	92	101
98/07/29	**5.75**	**18.39**	**30.29**	**3.93**	**0.93***	**20.87**	**0.36**	**2.09**	**2.00**	**.040**	**0.83**	**0.90**	**.47**	**92**	**99**
98/07/30	**5.75**	**18.39**	**30.29**	**3.38**	**0.84**	**21.34**	**0.36**	**1.91**	**2.00**	**.041**	**0.87**	**1.04**	**.47**	**92**	**99**
98/07/31	**5.75**	**18.39**	**30.29**	**2.79**	**0.84**	**21.67**	**0.42**	**2.04**	**2.00**	**.040**	**0.87**	**1.03**	**.47**	**92**	**100**
98/08/04	**5.75**	**18.39**	**30.29**	**1.12**	**0.93**	**22.56**	**0.45**	**1.65**	**2.00**	**.040**	**0.90**	**0.97**	**.47**	**92**	**101**

-terminated experiment

* biomass measured by filter

Growth of "adapted" ATCC 39676:pZB4L in 1% cCSL + Z salts at pH 5.75 & 30°C



C128 - 98/10/06-98/11/24

OPERATIONAL PARAMETERS FOR CONTINUOUS FERMENTATIONS

Sugar conversion by "adapted" *Zymomonas mobilis* ATCC 39676:pZB4L

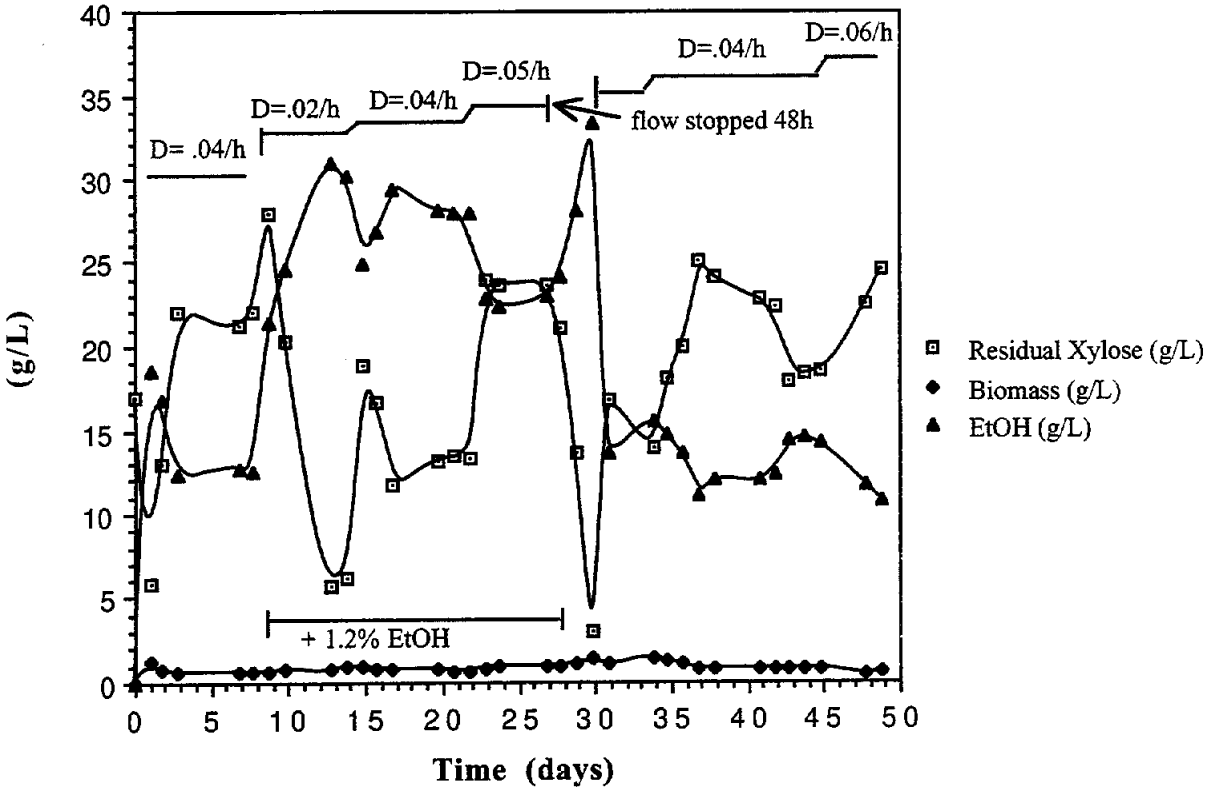
		SUBSTRATE			[PRODUCTS]					PRODUCTIVITY			YIELD		
Date	pH	[Glu] g/L	[Xyl] g/L	Residual [Xyl] (g/L)	Biomass g/L	EtOH g/L	Xylitol g/L?	Lactate g/L	Acetate g/L	D hr^{-1}	Q_p g P/L/h	q_p g P/g cell/h	$Y_{p/s}$ g P/g Glu	Conversion Efffic (%)	% C Recovery
C128- Medium=RM (flow started 24h after inoculation)															
98/10/07	5.75	25.88	16.98	5.90	1.20*	18.60	0.00	0.00	0.00	.000	-	-	.50	98	102
Medium=1%cCSL + 1.8mM $MgSO_4$ + 0.2% acetate															
98/10/08	5.75	18.22	30.07	12.98	0.75	16.80	0.00	1.56	2.0^	.041	0.69	0.92	.48	94	100
98/10/09	5.75	18.22	30.07	22.02	0.66	12.35	0.00	1.76	2.0^	.041	0.51	0.77	.47	92	101
98/10/13	**5.75**	**18.22**	**30.07**	**21.30**	**0.58**	**12.76**	**0.00**	**2.08**	**2.0^**	**.042**	**0.54**	**0.92**	**.47**	**92**	**102**
98/10/14	**5.75**	**18.22**	**30.07**	**22.02**	**0.58**	**12.48**	**0.00**	**2.42**	**2.0^**	**.038**	**0.47**	**0.82**	**.48**	**94**	**103**
Medium=1%cCSL + 1.8mM $MgSO_4$ + 0.2% acetate + 1.2% Ethanol															
98/10/15	5.75	18.50	31.07	27.95	0.58	21.44	0.00	3.19	2.0^	.020	0.20	0.34	.45	88	103
98/10/16	5.75	18.50	31.07	20.30	0.73	24.56	0.00	3.84	2.0^	.020	0.26	0.35	.44	86	101
98/10/19	5.75	18.50	31.07	5.78	0.73	30.98	0.42	6.38	2.0^	.020	0.39	0.53	.44	86	103
98/10/20	**5.75**	**18.64**	**30.58**	**6.17**	**0.96**	**30.22**	**0.07**	**6.44**	**2.0^**	**.020**	**0.37**	**0.39**	**.43**	**84**	**102**
98/10/21	5.75	18.64	30.58	18.94	0.96	24.90	0.00	4.63	2.0^	.040	0.53	0.55	.44	86	103
98/10/22	5.75	18.64	30.58	16.60	0.78	26.77	0.00	2.45	2.0^	.040	0.60	0.77	.46	90	100
98/10/23	5.75	18.64	30.58	11.78	0.81	29.44	0.00	2.21	2.0^	.040	0.71	0.88	.47	92	101
98/10/26	**5.75**	**18.52**	**30.23**	**13.25**	**0.81**	**28.08**	**0.00**	**2.05**	**2.0^**	**.042**	**0.71**	**0.87**	**.48**	**94**	**104**
98/10/27	**5.75**	**18.52**	**30.23**	**13.53**	**0.66**	**27.99**	**0.00**	**2.88**	**2.0^**	**.042**	**0.70**	**1.06**	**.48**	**94**	**103**
98/10/28	**5.75**	**18.52**	**30.23**	**13.39**	**0.66**	**27.96**	**0.00**	**2.89**	**2.0^**	**.043**	**0.72**	**1.09**	**.47**	**92**	**102**
98/10/29	5.75	18.52	30.23	23.89	0.84	22.80	0.00	2.34	2.0^	.054	0.49	0.58	.47	92	102
98/10/30	5.75	17.55	30.22	23.73	0.90	22.45	0.00	2.20	2.0^	.053	0.57	0.64	.45	88	101
98/11/02	**5.75**	**17.55**	**30.22**	**23.72**	**0.99**	**23.06**	**0.00**	**1.80**	**2.0^**	**.054**	**0.62**	**0.62**	**.48**	**94**	**103**
98/11/03	5.75	17.55	30.22	21.15	0.99	24.05	0.00	2.72	2.0^	.041	0.51	0.51	.47	92	103
-stopped flow for 48h															

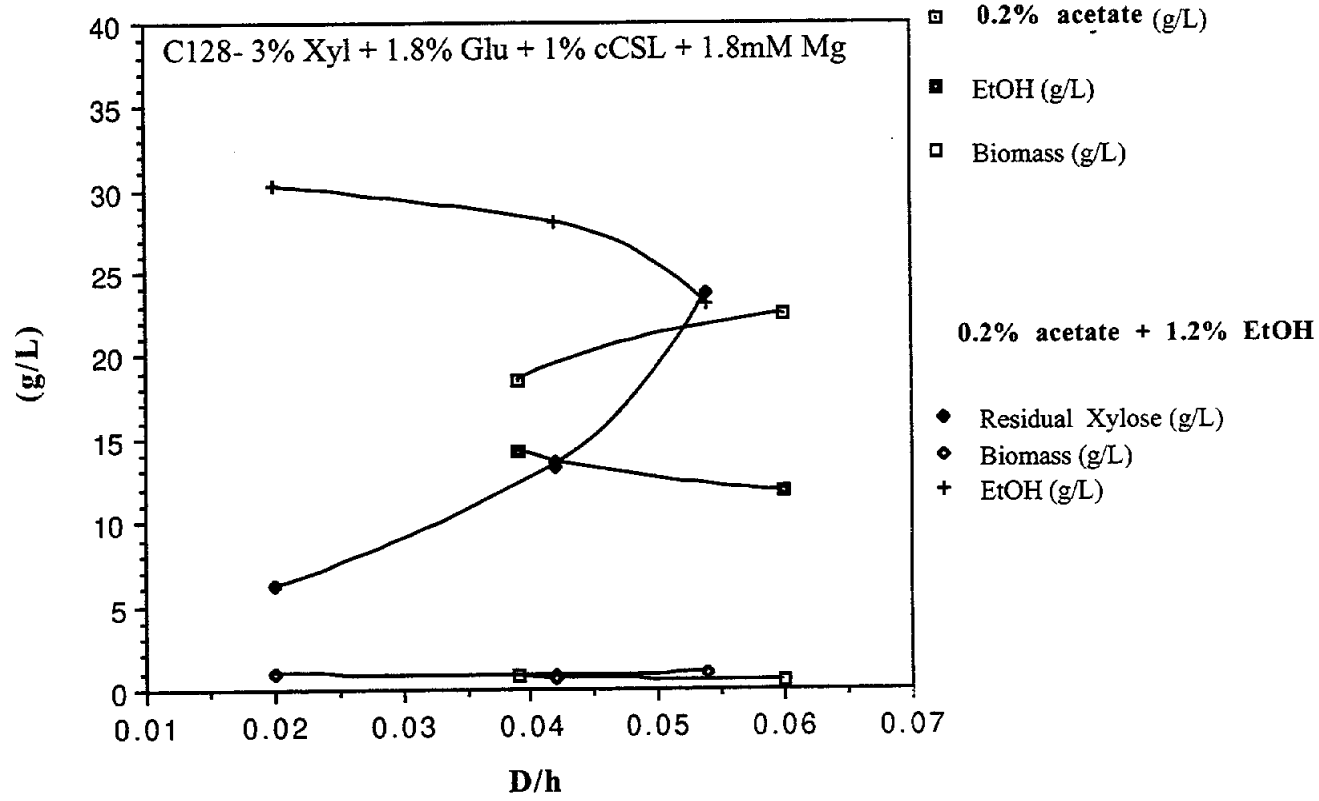
C128 - 98/10/06-98/11/24

		SUBSTRATE			[PRODUCTS]					PRODUCTIVITY			YIELD		
Date	pH	[Glu]	[Xyl]	Residual	Biomass	EtOH	Xylitol	Lactate	Acetate	D	Q_p	q_p	$Y_{p/s}$	Conversion	% C
		g/L	g/L	[Xyl] (g/L)	g/L	g/L	g/L?	g/L	g/L	hr^{-1}	g P/L/h	g P/g cell/h	g P/g Glu	Efffic (%)	Recovery
Medium=1%cCSL + 1.8mM $MgSO_4$ + 0.2% acetate															
98/11/04	5.75	17.55	30.22	13.68	1.17	28.04	0.00	2.76	2.0^	.000	-	-	.48	94	105
98/11/05	5.75	17.38	30.60	3.02	1.35	33.39	0.00	3.21	2.0^	.000	-	-	.48	94	104
98/11/06	5.75	17.38	30.60	16.76G	1.14	13.60	0.00	3.87	2.0^	.056	0.76	0.67	.47	92	107
98/11/09	5.75	17.38	30.60	14.02	1.43	15.56	0.58	4.35	2.0^	.028	0.44	0.30	.46	90	106
98/11/10	5.75	18.75	30.43	18.08	1.31	14.77	0.00	3.09	2.0^	.040	0.59	0.45	.47	92	105
98/11/11	5.75	18.75	30.43	19.96	1.05	13.60	0.00	1.04	2.0^	.039	0.53	0.51	.47	92	99
98/11/12	5.75	18.75	30.43	25.13	0.86	11.17	0.00	0.86	2.0^	.039	0.44	0.51	.46	90	99
98/11/13	**5.75**	**18.75**	**30.43**	**24.09**	**0.81**	**12.01**	**0.00**	**1.50**	**2.0^**	**.040**	**0.48**	**0.59**	**.48**	**94**	**102**
98/11/16	**5.75**	**18.75**	**30.43**	**22.82**	**0.78**	**12.01**	**0.00**	**1.65**	**2.0^**	**.040**	**0.48**	**0.62**	**.46**	**90**	**99**
98/11/17	**5.75**	**18.75**	**30.43**	**22.43**	**0.75**	**12.41**	**0.00**	**2.08**	**2.0^**	**.040**	**0.50**	**0.66**	**.46**	**90**	**101**
98/11/18	**5.75**	**18.75**	**30.43**	**17.98**	**0.75**	**14.48**	**0.00**	**1.55**	**2.0^**	**.042**	**0.61**	**0.81**	**.46**	**90**	**99**
98/11/19	**5.75**	**18.28**	**29.44**	**18.45**	**0.75**	**14.60**	**0.00**	**2.19**	**2.0^**	**.041**	**0.60**	**0.80**	**.50**	**98**	**105**
98/11/20	**5.75**	**18.28**	**29.44**	**18.50**	**0.75**	**14.22**	**0.00**	**1.83**	**2.0^**	**.039**	**0.55**	**0.74**	**.49**	**96**	**103**
98/11/23	**5.75**	**18.28**	**29.44**	**22.47**	**0.45**	**11.75**	**0.00**	**1.94**	**2.0^**	**.060**	**0.71**	**1.57**	**.47**	**92**	**100**
98/11/24	5.75	18.28	29.44	24.54	0.57	10.83	0.00	2.35	2.0^	.060	0.65	1.14	.47	92	102

-terminated experiment due to wall growth
* biomass measured by filter

C128 -1.8% glu & 3.0% Xyl-1%cCSL + 1.8mM Mg + 0.2% acetate ± 1.2% EtOH at pH 5.75 & 30°C





APPENDIX G

Summaries of chemostat experiments for Task 5

C120- 98/07/21 - 98/08/04

OPERATIONAL PARAMETERS FOR CONTINUOUS FERMENTATIONS

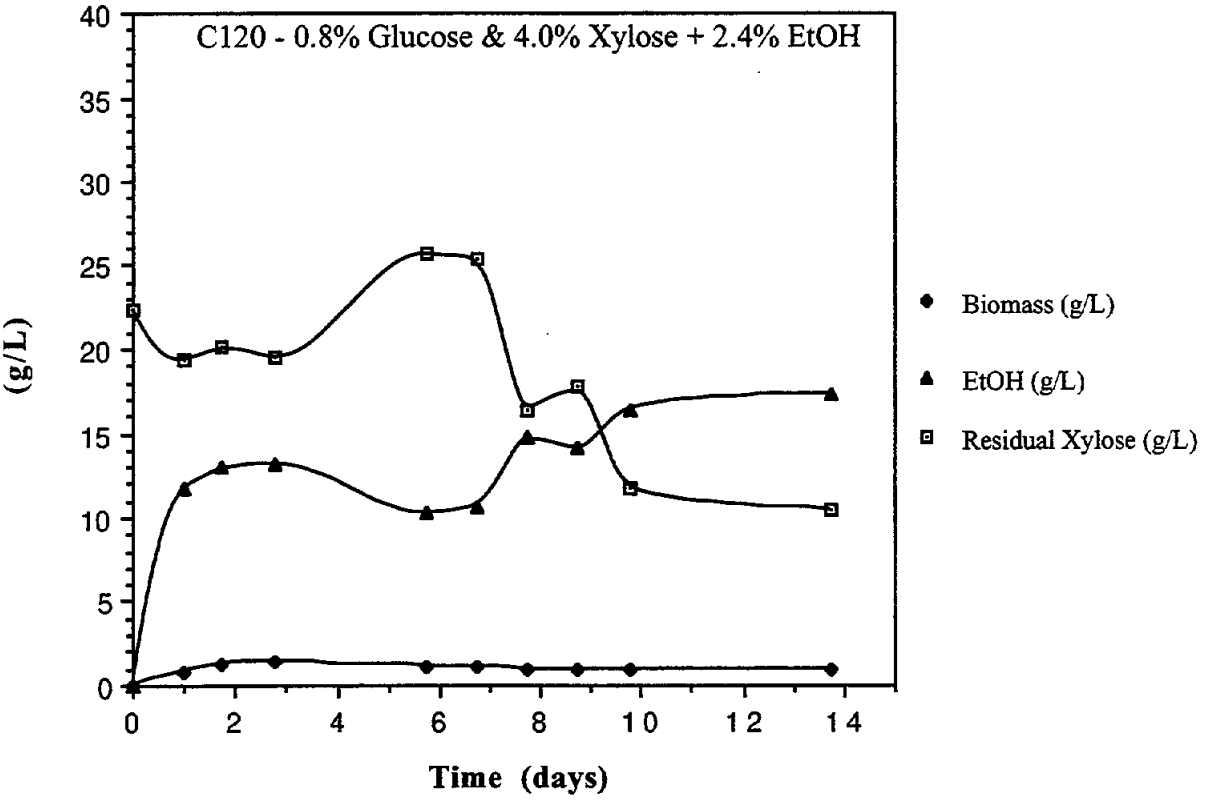
Sugar conversion by "adapted" *Zymomonas mobilis* ATCC 39676:pZB4L

		SUBSTRATE			[PRODUCTS]					PRODUCTIVITY			YIELD		
Date	pH	[Glu]	[Xyl]	Residual	Biomass	EtOH	Xylitol	Lactate	Acetate	D	Q_p	q_p	$Y_{p/s}$	Conversion	% C
		g/L	g/L	[Xyl] (g/L)	g/L	g/L	g/L?	g/L	g/L	hr^{-1}	g P/L/h	g P/g cell/h	g P/g Glu	Efffic (%)	Recovery
C120- Medium=ZM(flow started 24h after inoculation)															
98/07/22	5.75	20.36	22.46	19.29	0.87	11.77	0.00	0.00	0.00	.000	-	-	.50	98	102
Medium=1%cCSL(NACAN) +Zymo salts +2.4% EtOH															
98/07/23	5.75	7.39	39.33	20.10	1.29*	12.97	0.00	1.01	0.00	.032	0.42	0.32	.49	96	103
98/07/24	5.75	7.39	39.33	19.53	1.44	13.24	0.00	2.03	0.00	.037	0.49	0.34	.49	96	104
98/07/27	5.75	7.39	39.33	25.67	1.17	10.29	0.00	2.30	0.00	.038	0.39	0.33	.49	96	106
98/07/28	5.75	7.89	39.82	25.44	1.08	10.68	0.00	2.27	0.00	.041	0.44	0.41	.48	94	105
98/07/29	**5.75**	**7.89**	**39.82**	**16.41**	**1.02***	**14.71**	**0.00**	**2.47**	**0.00**	**.041**	**0.60**	**0.59**	**.47**	**92**	**102**
98/07/30	**5.75**	**7.89**	**39.82**	**17.72**	**0.90**	**14.06**	**0.00**	**2.12**	**0.00**	**.038**	**0.53**	**0.59**	**.47**	**92**	**102**
98/07/31	**5.75**	**7.89**	**39.82**	**11.76**	**0.90***	**16.41**	**0.00**	**1.80**	**0.00**	**.041**	**0.67**	**0.75**	**.46**	**90**	**98**
98/08/04	**5.75**	**7.89**	**39.82**	**10.52**	**0.99**	**17.33**	**0.00**	**2.23**	**0.00**	**.039**	**0.68**	**0.68**	**.47**	**92**	**100**

-terminated experiment

* biomass measured by filter

Growth of "adapted" ATCC 39676:pZB4L in 1% cCSL + Z salts at pH 5.75 & 30°C



C123- 98/09/15 - 98/10/02

OPERATIONAL PARAMETERS FOR CONTINUOUS FERMENTATIONS

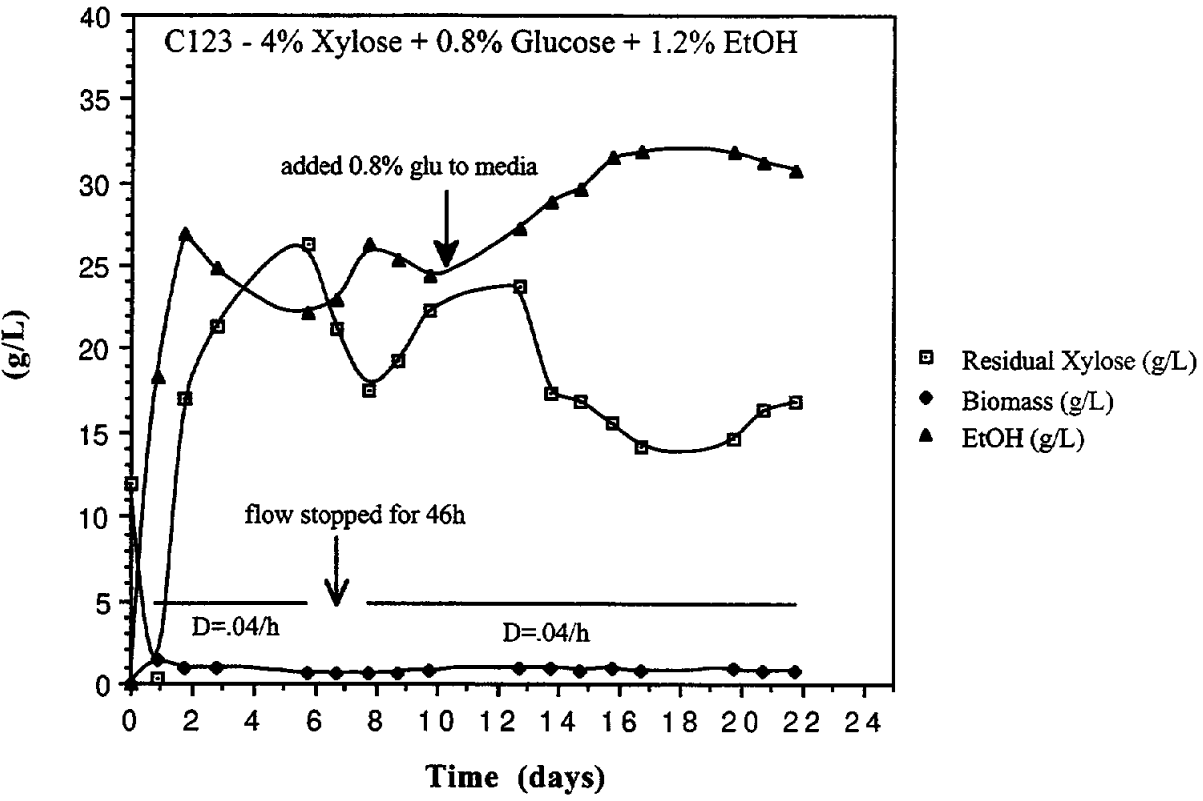
Sugar conversion by "adapted" *Zymomonas mobilis* ATCC 39676:pZB4L

		SUBSTRATE			[PRODUCTS]					PRODUCTIVITY			YIELD		
Date	pH	[Glu] g/L	[Xyl] g/L	Residual [Xyl] (g/L)	Biomass g/L	EtOH g/L	Xylitol g/L?	Lactate g/L	Acetate g/L	D hr^{-1}	Q_p g P/L/h	q_p g P/g cell/h	$Y_{p/s}$ g P/g Glu	Conversion Efffic (%)	% C Recovery
C123- Medium=RM(flow started 20h after inoculation)															
98/09/16	5.75	25.80	11.98	0.26	1.40*	18.29	0.00	0.00	0.00	.000	-	-	.49	96	100
Medium=1%cCSL + 1.8mM $MgSO_4$ + 1.2% EtOH															
98/09/17	5.75	8.53	39.45	17.11	1.00	26.96	0.00	0.93	0.00	.039	0.58	0.58	.48	94	101
98/09/18	5.75	8.53	39.45	21.33	1.02	24.82	0.00	3.44	0.00	.040	0.51	0.50	.48	94	106
98/09/21	**5.75**	**8.53**	**39.45**	**26.33**	**0.65**	**22.19**	**0.00**	**1.29**	**0.00**	**.039**	**0.40**	**0.61**	**.47**	**92**	**101**
-stopped flow for 46hours															
98/09/22	5.75	8.53	39.45	21.12G	0.60	22.91	0.00	2.37	0.00	.000	-	-	.46	90	102
98/09/23	5.75	8.53	39.45	17.57	0.69	26.26	0.00	1.07	0.00	.000	-	-	.47	92	99
98/09/24	5.75	8.53	39.45	19.32	0.69	25.39	0.00	2.04	0.00	.041	0.55	0.80	.47	92	101
98/09/25	5.75	8.53	39.45	22.27	0.80	24.46	0.00	1.59	0.00	.040	0.50	0.62	.48	94	103
98/09/28	**5.75**	**15.88**	**39.95**	**23.76**	**1.01***	**27.32**	**0.00**	**3.30**	**0.00**	**.040**	**0.60**	**0.60**	**.47**	**92**	**103**
pH increased to pH10 for a short time-stopped flow for 8 hours															
98/09/29	5.75	15.88	39.92	17.34G	1.01	28.89	0.00	3.15	0.00	.040	0.67	0.66	.46	90	102
98/09/30	5.75	15.88	39.92	16.92G	0.77	29.59	0.00	3.49	0.00	.039	0.68	0.88	.48	94	103
98/10/01	5.75	15.88	39.92	15.54	0.96	31.49	0.00	3.14	0.00	.039	0.75	0.78	.48	94	103
98/10/02	**5.75**	**15.88**	**39.92**	**14.21**	**0.77**	**31.82**	**0.00**	**2.59**	**0.00**	**.039**	**0.76**	**0.99**	**.47**	**92**	**100**
98/10/05	5.75	15.83	40.61	14.59	0.89	31.81	0.00	2.45	0.00	.024	0.47	0.53	.47	92	101
98/10/06	5.75	15.83	40.61	16.39	0.77	31.26	0.00	2.47	0.00	.040	0.76	0.99	.47	92	101
98/10/07	5.75	15.83	40.61	16.83	0.80	30.78	0.00	2.50	0.00	.040	0.74	0.93	.47	92	100

-contaminated reservoir, terminated experiment

* biomass measured by filter

Growth of "adapted" ATCC 39676:pZB4L in 1% cCSL + 1.8mM Mg + 1.2% EtOH at pH 5.75 & 30°C



C126 - 98/09/15-98/10/07

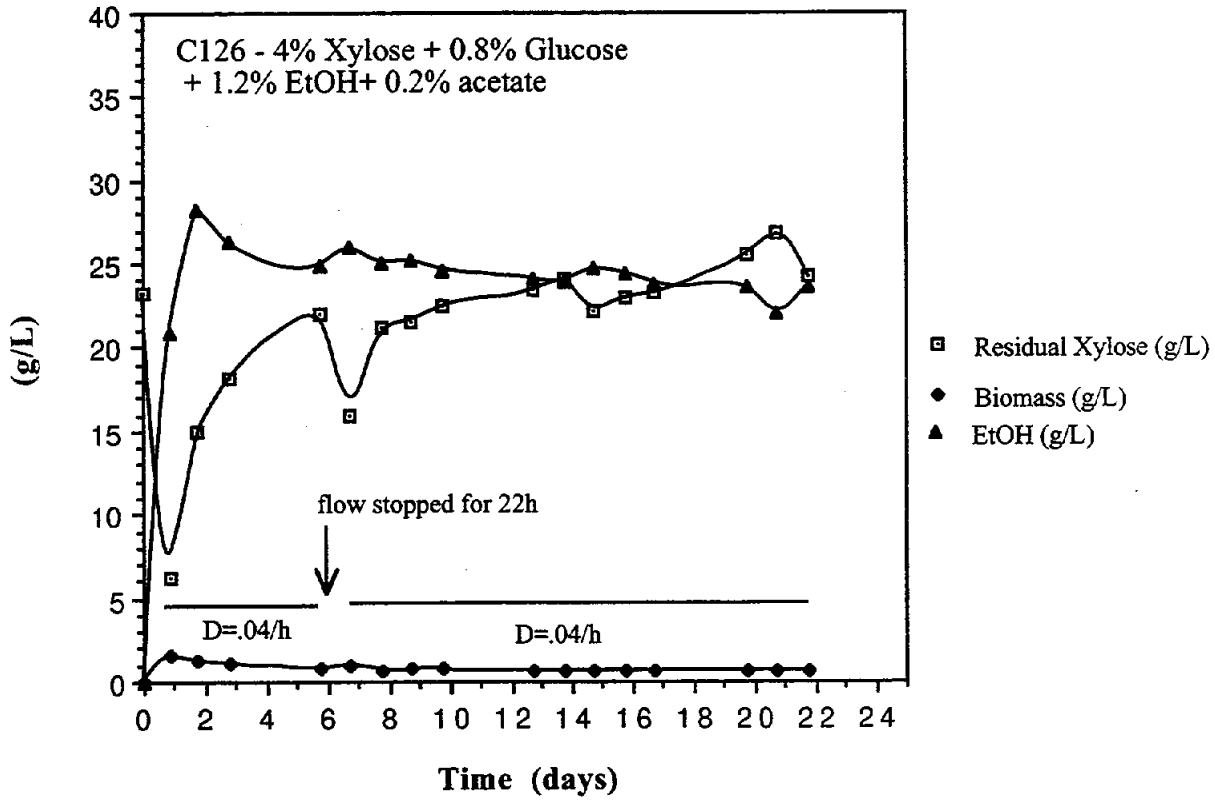
OPERATIONAL PARAMETERS FOR CONTINUOUS FERMENTATIONS

Sugar conversion by "adapted" *Zymomonas mobilis* ATCC 39676:pZB4L

		SUBSTRATE			[PRODUCTS]					PRODUCTIVITY			YIELD		
Date	pH	[Glu]	[Xyl]	Residual	Biomass	EtOH	Xylitol	Lactate	Acetate	D	Q_p	q_p	$Y_{p/s}$	Conversion	% C
		g/L	g/L	[Xyl] (g/L)	g/L	g/L	g/L?	g/L	g/L	hr^{-1}	g P/L/h	g P/g cell/h	g P/g Glu	Efffic (%)	Recovery
C126- Medium=RM (flow started 20h after inoculation)															
98/09/16	5.75	26.73	23.29	6.28	1.60*	20.94	0.00	0.00	0.00	.000	-	-	.48	94	98
Medium=1%cCSL + 1.8mM $MgSO_4$ + 0.2% acetate + 1.2% EtOH															
98/09/17	5.75	8.34	40.95	14.94	1.23	28.26	0.00	1.70	2.0^	.041	0.67	0.54	.47	92	101
98/09/18	5.75	8.34	40.95	18.14	1.08	26.22	0.00	2.71	2.0^	.040	0.57	0.53	.46	90	101
98/09/21	**5.75**	**8.34**	**40.95**	**22.00**	**0.81**	**24.88**	**0.00**	**2.75**	**2.0^**	**.040**	**0.52**	**0.64**	**.47**	**92**	**103**
-stopped flow for 22 hours															
98/09/22	5.75	8.34	40.95	16.00	0.93	26.02	0.00	4.08	2.0^	.000	-	-	.45	88	103
98/09/23	5.75	8.34	40.95	21.15	0.69	25.02	0.00	2.33	2.0^	.040	0.52	0.75	.46	90	101
98/09/24	5.75	8.34	40.95	21.57	0.72	25.16	0.00	1.73	2.0^	.040	0.53	0.73	.47	92	101
98/09/25	5.75	8.34	40.95	22.41	0.72	24.60	0.00	2.15	2.0^	.040	0.50	0.70	.47	92	102
98/09/28	**5.75**	**8.34**	**40.95**	**23.43**	**0.58***	**24.05**	**0.00**	**1.62**	**2.0^**	**.040**	**0.48**	**0.83**	**.47**	**92**	**100**
98/09/29	**5.75**	**8.34**	**40.95**	**24.02**	**0.58**	**23.87**	**0.00**	**2.10**	**2.0^**	**.040**	**0.47**	**0.82**	**.47**	**92**	**102**
98/09/30	**5.75**	**8.34**	**40.95**	**22.12**	**0.64**	**24.66**	**0.00**	**2.76**	**2.0^**	**.040**	**0.51**	**0.79**	**.47**	**92**	**102**
98/10/01	**5.75**	**8.34**	**40.95**	**22.92**	**0.58***	**24.44**	**0.00**	**3.24**	**2.0^**	**.040**	**0.50**	**0.86**	**.47**	**92**	**104**
98/10/02	**5.75**	**8.34**	**40.95**	**23.27**	**0.58**	**23.80**	**0.00**	**3.16**	**2.0^**	**.040**	**0.47**	**0.81**	**.45**	**88**	**102**
98/10/05	**5.75**	**8.34**	**40.95**	**25.50**	**0.60**	**23.57**	**0.00**	**3.85**	**2.0^**	**.040**	**0.46**	**0.77**	**.49**	**96**	**107**
98/10/06	**5.75**	**8.34**	**40.95**	**26.78**	**0.58**	**22.02**	**0.00**	**3.74**	**2.0^**	**.040**	**0.40**	**0.69**	**.45**	**88**	**103**
98/10/07	**5.75**	**8.34**	**40.95**	**24.28**	**0.58**	**23.63**	**0.00**	**3.95**	**2.0^**	**.040**	**0.47**	**0.80**	**.47**	**92**	**105**

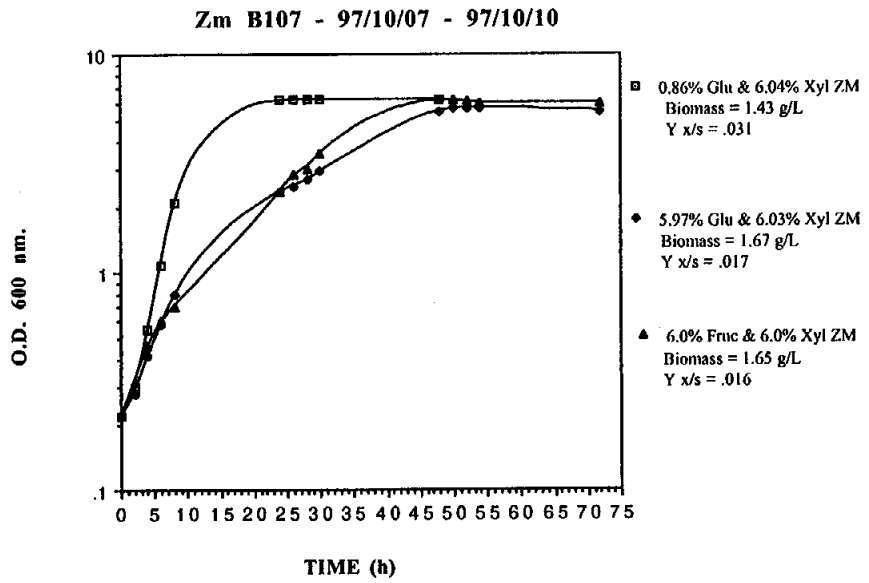
-terminated experiment
* biomass measured by filter
^0.2% acetate added to media

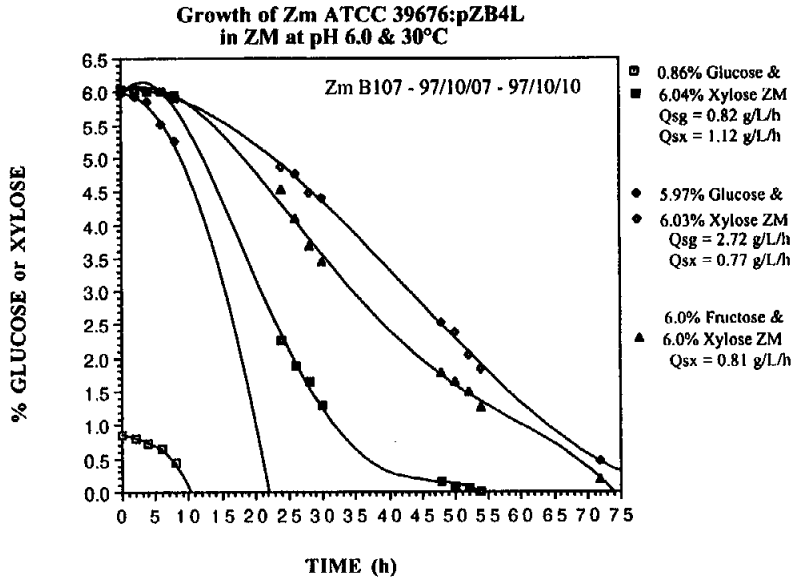
Growth of "adapted" ATCC 39676:pZB4L in 1% cCSL + 1.8mM Mg + 1.2% EtOH + 0.2% acetate at pH 5.75 & 30°C

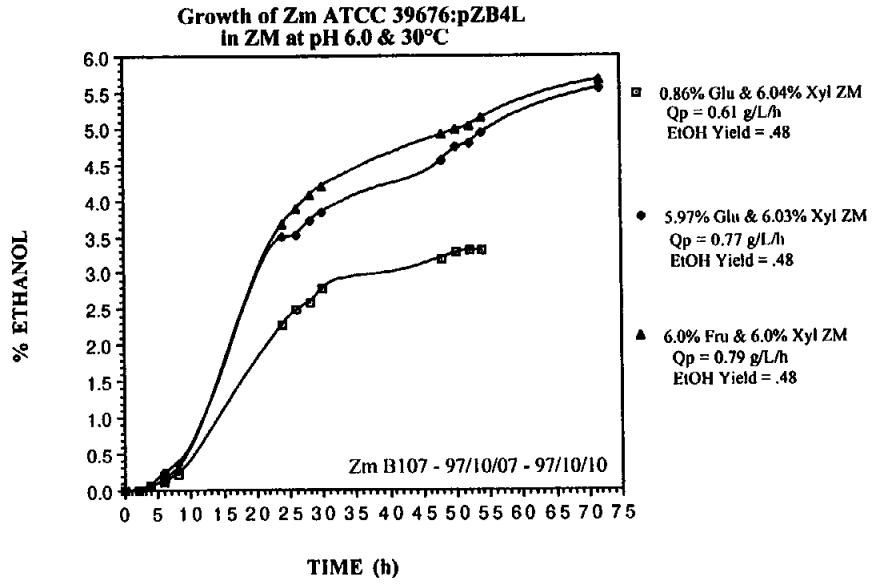


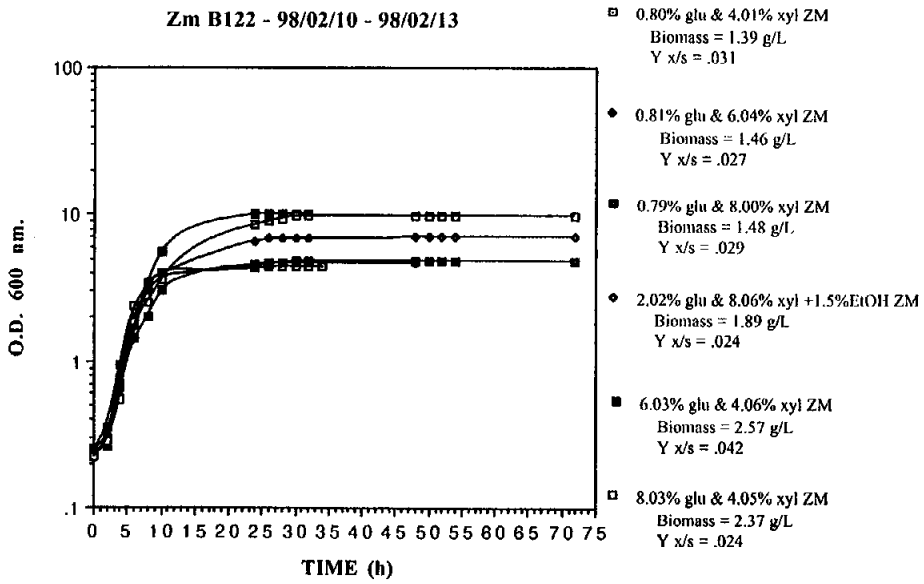
APPENDIX H

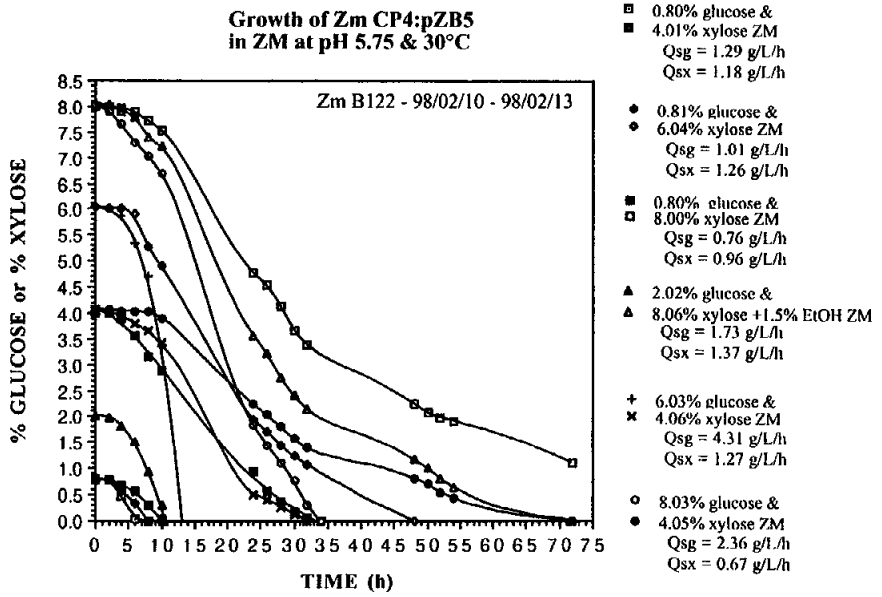
Graphical summaries for subcontract extension Task 3

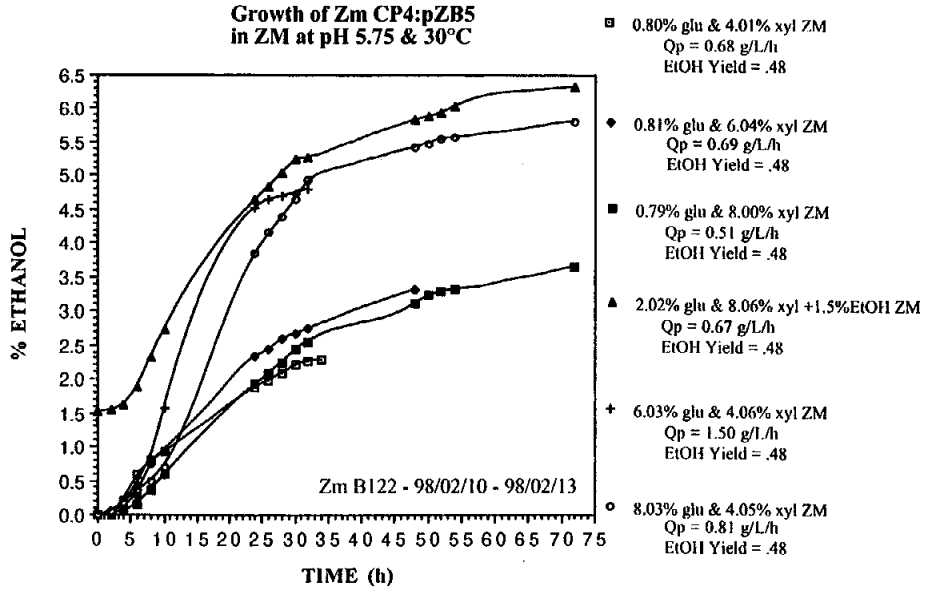

Zm B107 - 97/10/07 - 97/10/10
O.D. 600 nm.
10
1
.1
0 5 10 15 20 25 30 35 40 45 50 55 60 65 70 75
TIME (h)
0.86% Glu & 6.04% Xyl ZM
Biomass = 1.43 g/L
Y x/s = .031
5.97% Glu & 6.03% Xyl ZM
Biomass = 1.67 g/L
Y x/s = .017
6.0% Fruc & 6.0% Xyl ZM
Biomass = 1.65 g/L
Y x/s = .016

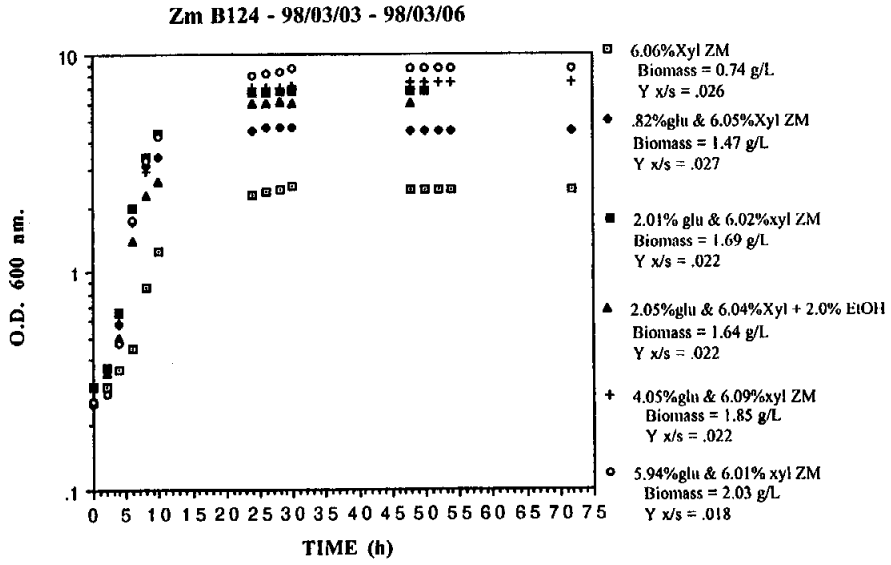

Growth of Zm ATCC 39676:pZB4L
in ZM at pH 6.0 & 30°C
Zm B107 - 97/10/07 - 97/10/10
% GLUCOSE or XYLOSE
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
0 5 10 15 20 25 30 35 40 45 50 55 60 65 70 75
TIME (h)
0.86% Glucose &
6.04% Xylose ZM
Qsg = 0.82 g/L/h
Qsx = 1.12 g/L/h
5.97% Glucose &
6.03% Xylose ZM
Qsg = 2.72 g/L/h
Qsx = 0.77 g/L/h
6.0% Fructose &
6.0% Xylose ZM
Qsx = 0.81 g/L/h

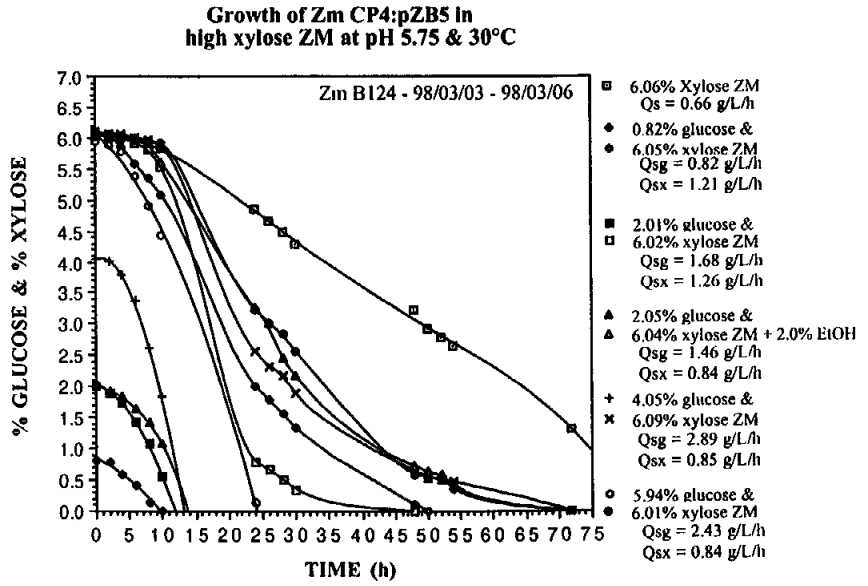

Growth of Zm ATCC 39676:pZB4L
in ZM at pH 6.0 & 30°C
% ETHANOL
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
Zm B107 - 97/10/07 - 97/10/10
0 5 10 15 20 25 30 35 40 45 50 55 60 65 70 75
TIME (h)
0.86% Glu & 6.04% Xyl ZM
Qp = 0.61 g/L/h
EtOH Yield = .48
5.97% Glu & 6.03% Xyl ZM
Qp = 0.77 g/L/h
EtOH Yield = .48
6.0% Fru & 6.0% Xyl ZM
Qp = 0.79 g/L/h
EtOH Yield = .48

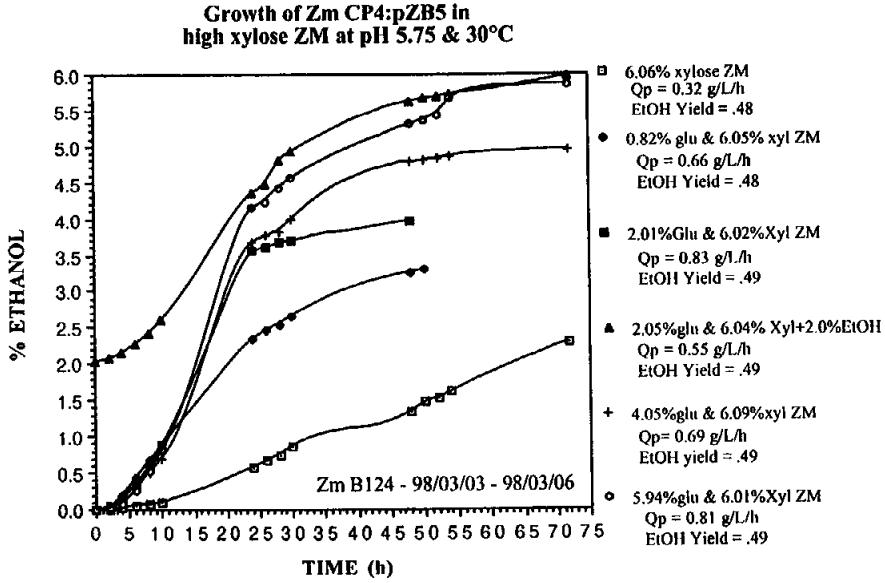

Zm B122 - 98/02/10 - 98/02/13
O.D. 600 nm.
100
10
1
.1
0 5 10 15 20 25 30 35 40 45 50 55 60 65 70 75
TIME (h)
0.80% glu & 4.01% xyl ZM
Biomass = 1.39 g/L
Y x/s = .031
0.81% glu & 6.04% xyl ZM
Biomass = 1.46 g/L
Y x/s = .027
0.79% glu & 8.00% xyl ZM
Biomass = 1.48 g/L
Y x/s = .029
2.02% glu & 8.06% xyl +1.5%EtOH ZM
Biomass = 1.89 g/L
Y x/s = .024
6.03% glu & 4.06% xyl ZM
Biomass = 2.57 g/L
Y x/s = .042
8.03% glu & 4.05% xyl ZM
Biomass = 2.37 g/L
Y x/s = .024

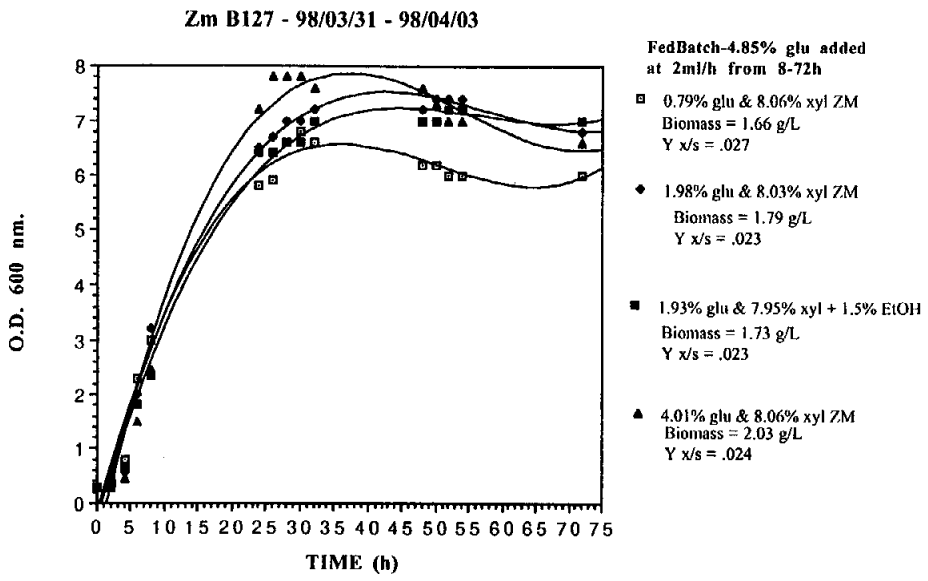

Growth of Zm CP4:pZB5
in ZM at pH 5.75 & 30°C
Zm B122 - 98/02/10 - 98/02/13
% GLUCOSE or % XYLOSE
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
0 5 10 15 20 25 30 35 40 45 50 55 60 65 70 75
TIME (h)
0.80% glucose &
4.01% xylose ZM
Qsg = 1.29 g/L/h
Qsx = 1.18 g/L/h
0.81% glucose &
6.04% xylose ZM
Qsg = 1.01 g/L/h
Qsx = 1.26 g/L/h
0.80% glucose &
8.00% xylose ZM
Qsg = 0.76 g/L/h
Qsx = 0.96 g/L/h
2.02% glucose &
8.06% xylose +1.5% EtOH ZM
Qsg = 1.73 g/L/h
Qsx = 1.37 g/L/h
6.03% glucose &
4.06% xylose ZM
Qsg = 4.31 g/L/h
Qsx = 1.27 g/L/h
8.03% glucose &
4.05% xylose ZM
Qsg = 2.36 g/L/h
Qsx = 0.67 g/L/h

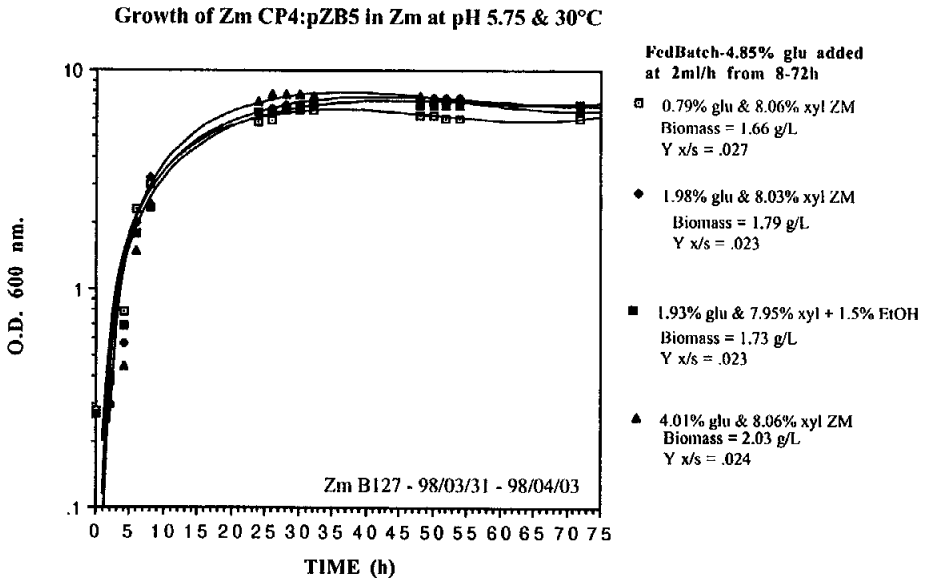

Growth of Zm CP4:pZB5
in ZM at pH 5.75 & 30°C
% ETHANOL
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
Zm B122 - 98/02/10 - 98/02/13
0 5 10 15 20 25 30 35 40 45 50 55 60 65 70 75
TIME (h)
0.80% glu & 4.01% xyl ZM
Qp = 0.68 g/L/h
EtOH Yield = .48
0.81% glu & 6.04% xyl ZM
Qp = 0.69 g/L/h
EtOH Yield = .48
0.79% glu & 8.00% xyl ZM
Qp = 0.51 g/L/h
EtOH Yield = .48
2.02% glu & 8.06% xyl +1.5%EtOH ZM
Qp = 0.67 g/L/h
EtOH Yield = .48
6.03% glu & 4.06% xyl ZM
Qp = 1.50 g/L/h
EtOH Yield = .48
8.03% glu & 4.05% xyl ZM
Qp = 0.81 g/L/h
EtOH Yield = .48

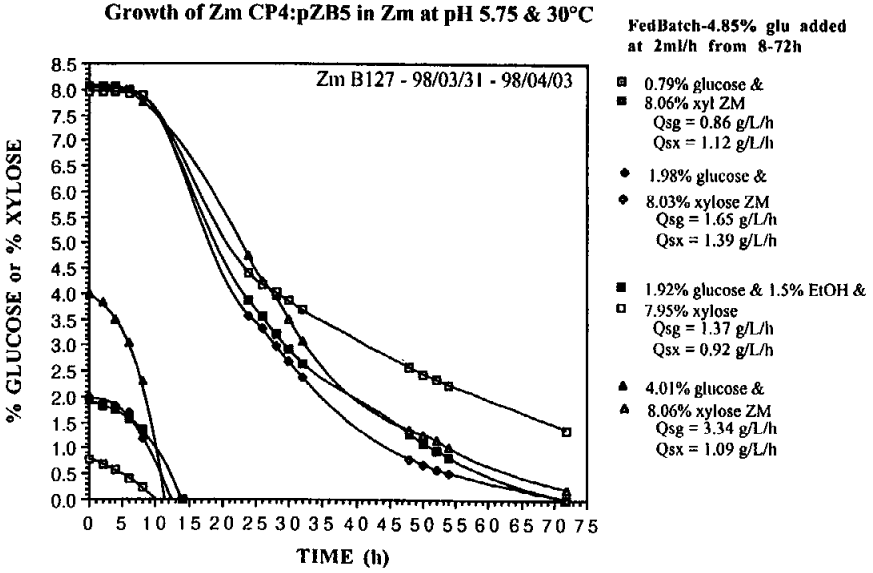

Zm B124 - 98/03/03 - 98/03/06
O.D. 600 nm.
TIME (h)
6.06%Xyl ZM
Biomass = 0.74 g/L
Y x/s = .026
.82%glu & 6.05%Xyl ZM
Biomass = 1.47 g/L
Y x/s = .027
2.01% glu & 6.02%xyl ZM
Biomass = 1.69 g/L
Y x/s = .022
2.05%glu & 6.04%Xyl + 2.0% EtOH
Biomass = 1.64 g/L
Y x/s = .022
4.05%glu & 6.09%xyl ZM
Biomass = 1.85 g/L
Y x/s = .022
5.94%glu & 6.01% xyl ZM
Biomass = 2.03 g/L
Y x/s = .018

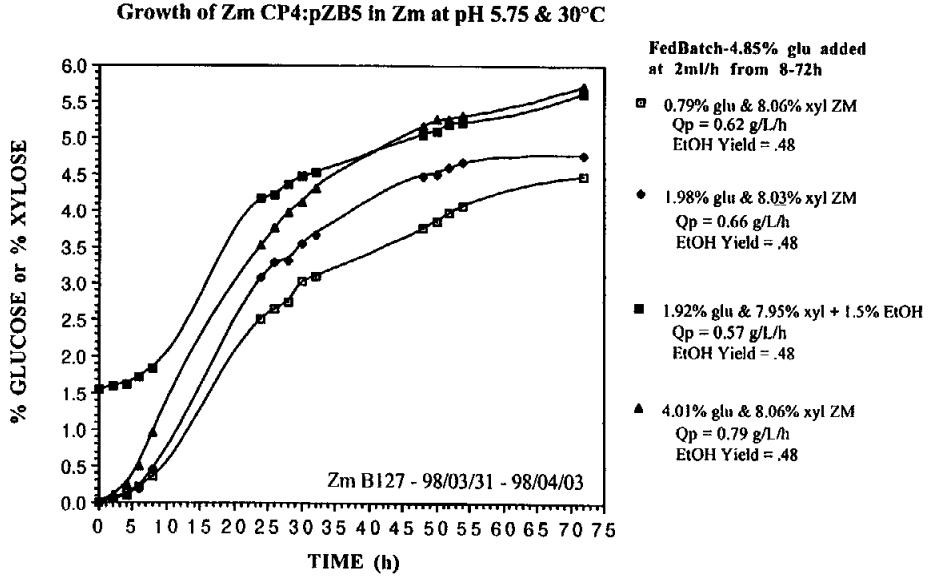

Growth of Zm CP4:pZB5 in
high xylose ZM at pH 5.75 & 30°C
Zm B124 - 98/03/03 - 98/03/06
% GLUCOSE & % XYLOSE
TIME (h)
6.06% Xylose ZM
Qs = 0.66 g/L/h
0.82% glucose &
6.05% xylose ZM
Qsg = 0.82 g/L/h
Qsx = 1.21 g/L/h
2.01% glucose &
6.02% xylose ZM
Qsg = 1.68 g/L/h
Qsx = 1.26 g/L/h
2.05% glucose &
6.04% xylose ZM + 2.0% EtOH
Qsg = 1.46 g/L/h
Qsx = 0.84 g/L/h
4.05% glucose &
6.09% xylose ZM
Qsg = 2.89 g/L/h
Qsx = 0.85 g/L/h
5.94% glucose &
6.01% xylose ZM
Qsg = 2.43 g/L/h
Qsx = 0.84 g/L/h

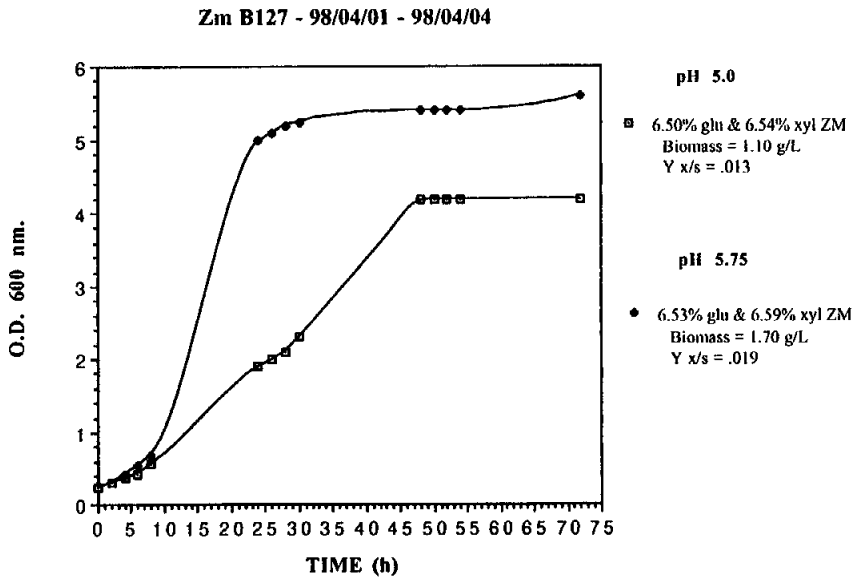

Growth of Zm CP4:pZB5 in
high xylose ZM at pH 5.75 & 30°C
% ETHANOL
TIME (h)
Zm B124 - 98/03/03 - 98/03/06
6.06% xylose ZM
Qp = 0.32 g/L/h
EtOH Yield = .48
0.82% glu & 6.05% xyl ZM
Qp = 0.66 g/L/h
EtOH Yield = .48
2.01%Glu & 6.02%Xyl ZM
Qp = 0.83 g/L/h
EtOH Yield = .49
2.05%glu & 6.04% Xyl+2.0%EtOH
Qp = 0.55 g/L/h
EtOH Yield = .49
4.05%glu & 6.09%xyl ZM
Qp= 0.69 g/L/h
EtOH yield = .49
5.94%glu & 6.01%Xyl ZM
Qp = 0.81 g/L/h
EtOH Yield = .49

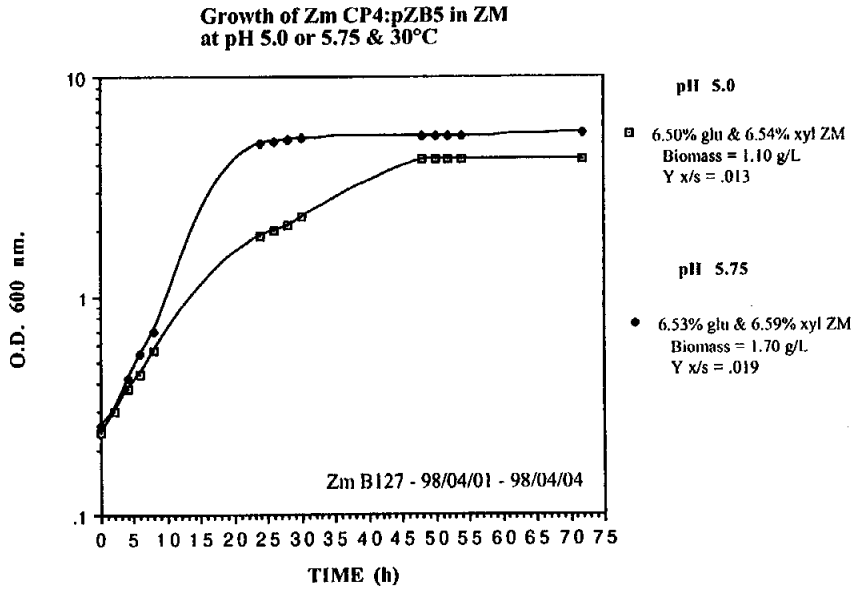

Zm B127 - 98/03/31 - 98/04/03
O.D. 600 nm.
TIME (h)
FedBatch-4.85% glu added at 2ml/h from 8-72h
0.79% glu & 8.06% xyl ZM
Biomass = 1.66 g/L
Y x/s = .027
1.98% glu & 8.03% xyl ZM
Biomass = 1.79 g/L
Y x/s = .023
1.93% glu & 7.95% xyl + 1.5% EtOH
Biomass = 1.73 g/L
Y x/s = .023
4.01% glu & 8.06% xyl ZM
Biomass = 2.03 g/L
Y x/s = .024

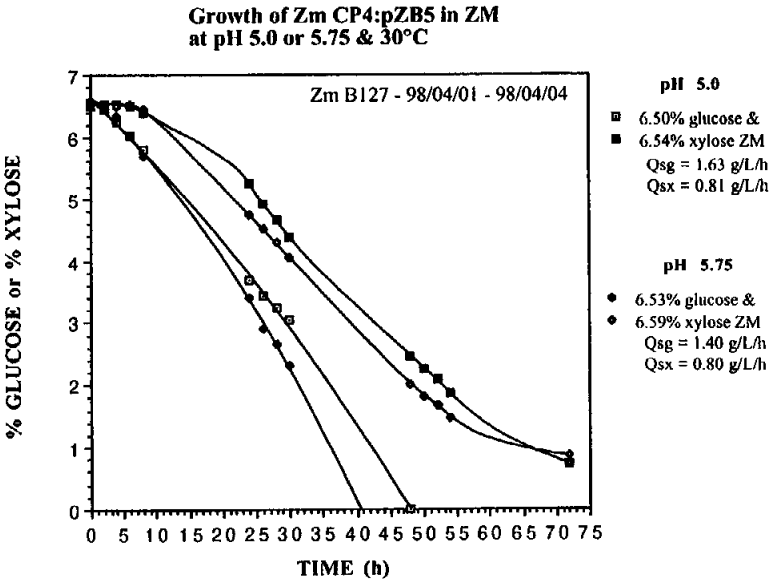

Growth of Zm CP4:pZB5 in Zm at pH 5.75 & 30°C
O.D. 600 nm.
TIME (h)
Zm B127 - 98/03/31 - 98/04/03
FedBatch-4.85% glu added at 2ml/h from 8-72h
0.79% glu & 8.06% xyl ZM
Biomass = 1.66 g/L
Y x/s = .027
1.98% glu & 8.03% xyl ZM
Biomass = 1.79 g/L
Y x/s = .023
1.93% glu & 7.95% xyl + 1.5% EtOH
Biomass = 1.73 g/L
Y x/s = .023
4.01% glu & 8.06% xyl ZM
Biomass = 2.03 g/L
Y x/s = .024

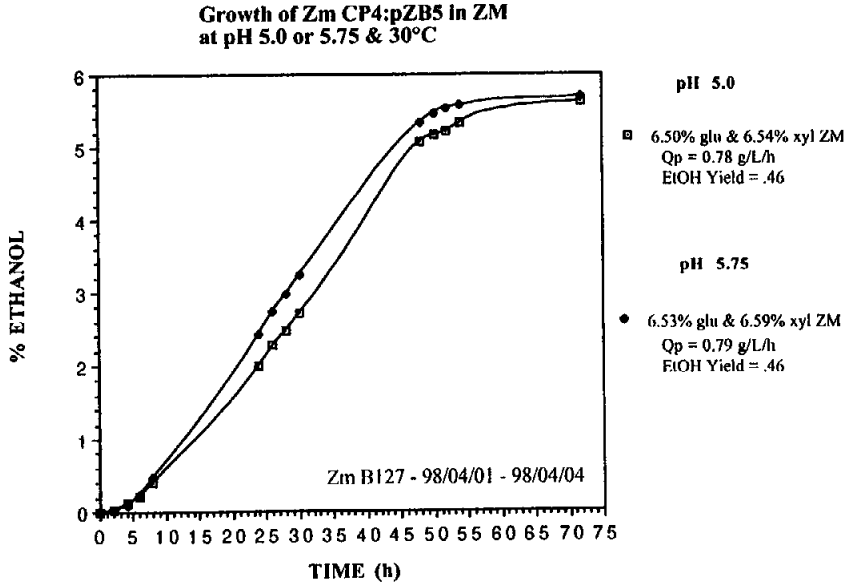

Growth of Zm CP4:pZB5 in Zm at pH 5.75 & 30°C
% GLUCOSE or % XYLOSE
TIME (h)
Zm B127 - 98/03/31 - 98/04/03
FedBatch-4.85% glu added at 2ml/h from 8-72h
0.79% glucose & 8.06% xyl ZM
Qsg = 0.86 g/L/h
Qsx = 1.12 g/L/h
1.98% glucose & 8.03% xylose ZM
Qsg = 1.65 g/L/h
Qsx = 1.39 g/L/h
1.92% glucose & 1.5% EtOH & 7.95% xylose
Qsg = 1.37 g/L/h
Qsx = 0.92 g/L/h
4.01% glucose & 8.06% xylose ZM
Qsg = 3.34 g/L/h
Qsx = 1.09 g/L/h


Growth of Zm CP4:pZB5 in Zm at pH 5.75 & 30°C
% GLUCOSE or % XYLOSE
TIME (h)
Zm B127 - 98/03/31 - 98/04/03
FedBatch-4.85% glu added at 2ml/h from 8-72h
0.79% glu & 8.06% xyl ZM
Qp = 0.62 g/L/h
EtOH Yield = .48
1.98% glu & 8.03% xyl ZM
Qp = 0.66 g/L/h
EtOH Yield = .48
1.92% glu & 7.95% xyl + 1.5% EtOH
Qp = 0.57 g/L/h
EtOH Yield = .48
4.01% glu & 8.06% xyl ZM
Qp = 0.79 g/L/h
EtOH Yield = .48

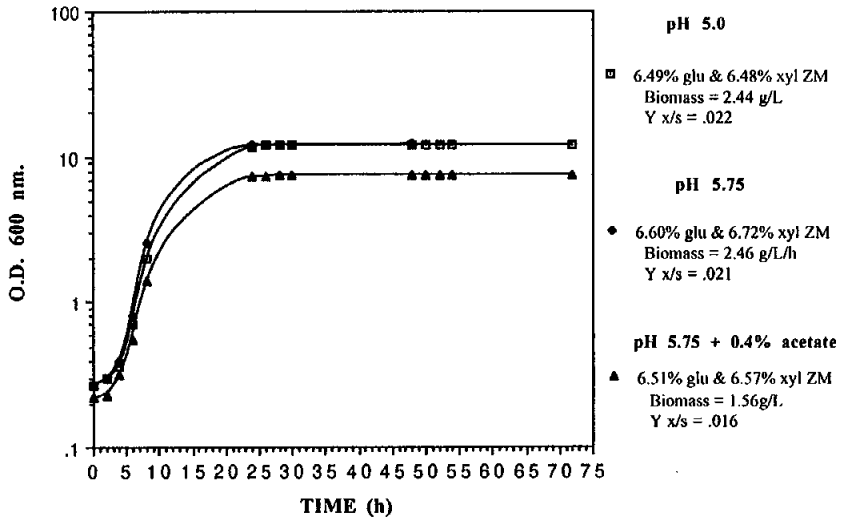

Zm B127 - 98/04/01 - 98/04/04
O.D. 600 nm.
TIME (h)
pH 5.0
6.50% glu & 6.54% xyl ZM
Biomass = 1.10 g/L
Y x/s = .013
pH 5.75
6.53% glu & 6.59% xyl ZM
Biomass = 1.70 g/L
Y x/s = .019


Growth of Zm CP4:pZB5 in ZM
at pH 5.0 or 5.75 & 30°C
O.D. 600 nm.
TIME (h)
Zm B127 - 98/04/01 - 98/04/04
pH 5.0
6.50% glu & 6.54% xyl ZM
Biomass = 1.10 g/L
Y x/s = .013
pH 5.75
6.53% glu & 6.59% xyl ZM
Biomass = 1.70 g/L
Y x/s = .019

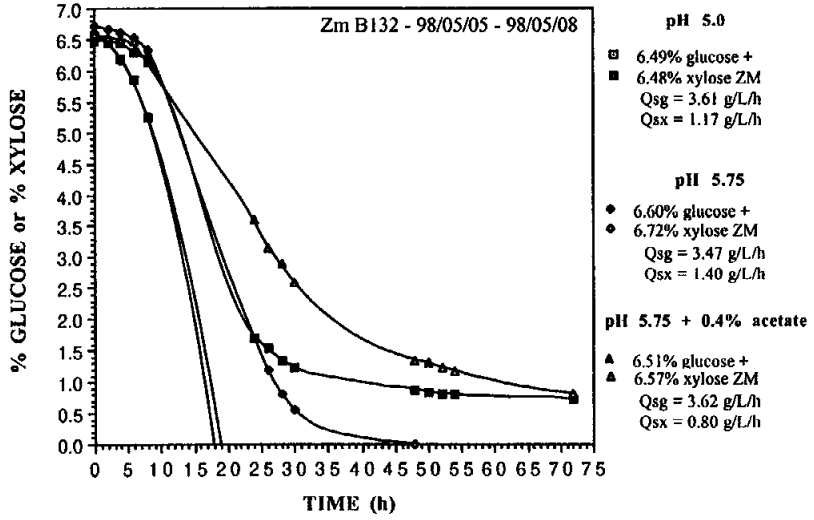

Growth of Zm CP4:pZB5 in ZM
at pH 5.0 or 5.75 & 30°C
% GLUCOSE or % XYLOSE
TIME (h)
Zm B127 - 98/04/01 - 98/04/04
pH 5.0
6.50% glucose &
6.54% xylose ZM
Qsg = 1.63 g/L/h
Qsx = 0.81 g/L/h
pH 5.75
6.53% glucose &
6.59% xylose ZM
Qsg = 1.40 g/L/h
Qsx = 0.80 g/L/h


Growth of Zm CP4:pZB5 in ZM
at pH 5.0 or 5.75 & 30°C
% ETHANOL
TIME (h)
Zm B127 - 98/04/01 - 98/04/04
pH 5.0
6.50% glu & 6.54% xyl ZM
Qp = 0.78 g/L/h
EtOH Yield = .46
pH 5.75
6.53% glu & 6.59% xyl ZM
Qp = 0.79 g/L/h
EtOH Yield = .46

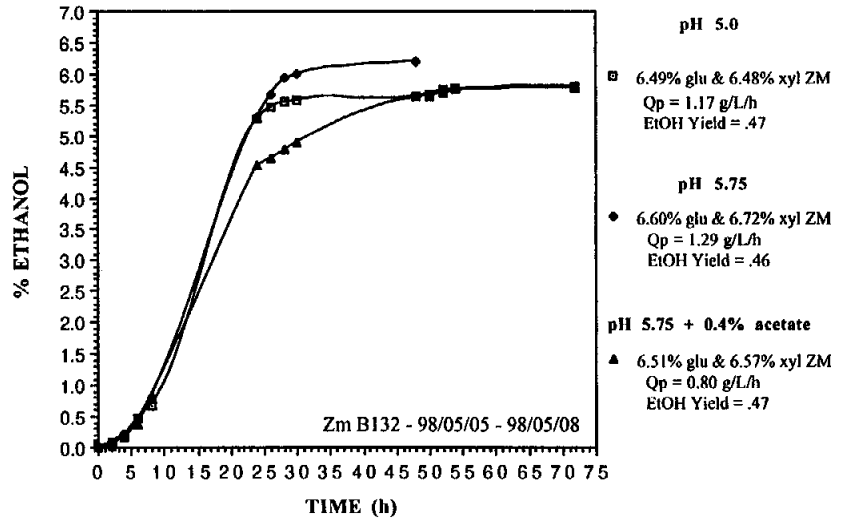
Zm B132 - 98/05/05 - 98/05/08

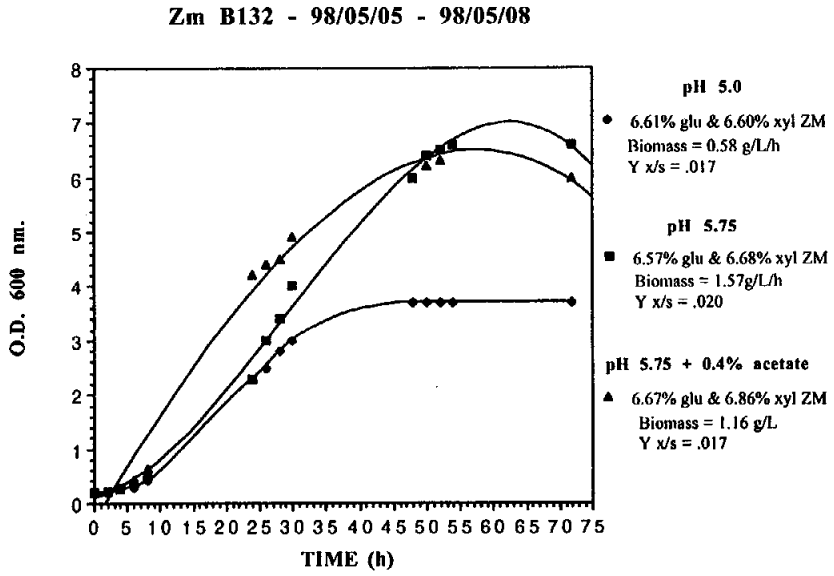


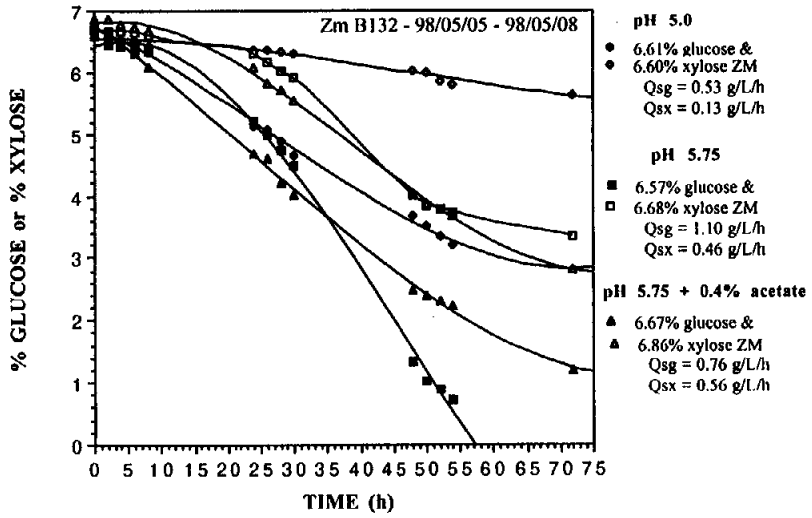
Growth of CP4:pZB5 in 6.5% glucose + 6.5% xylose at pH 5.0 or pH 5.75 ± 0.4% acetate at 30°C

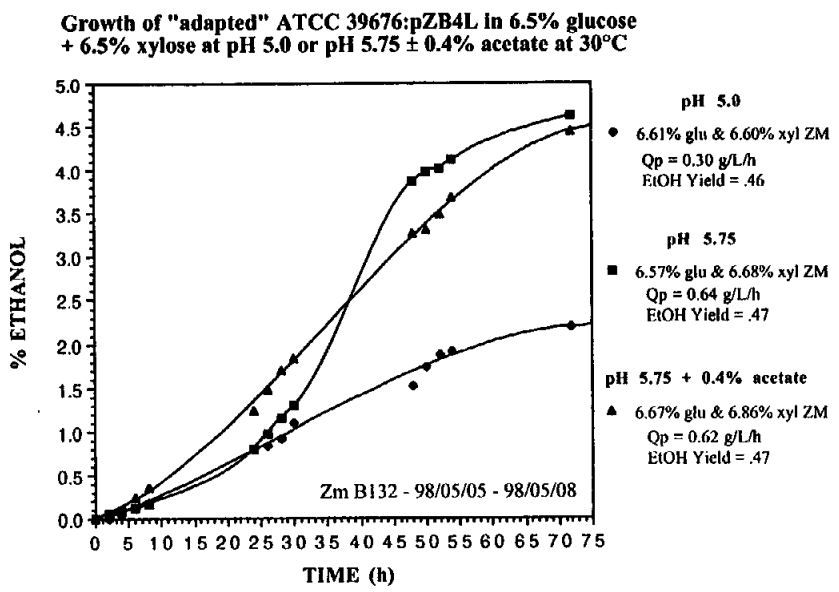


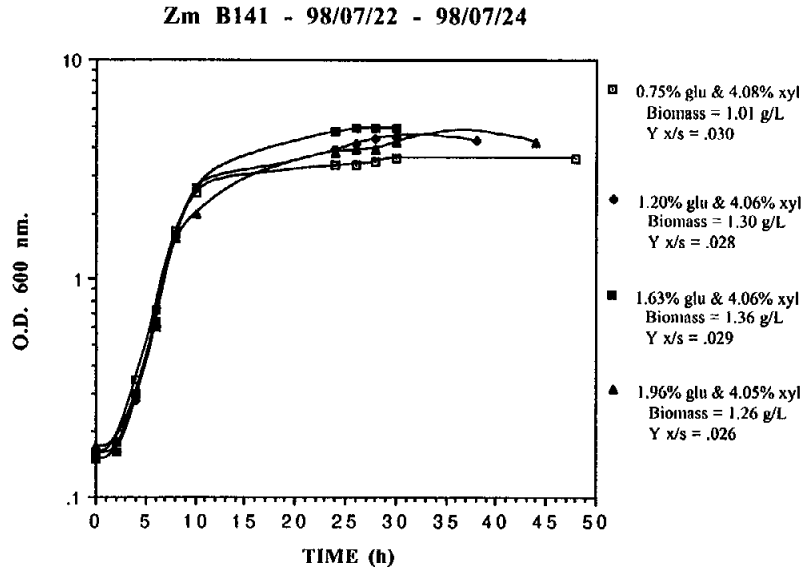
Growth of CP4:pZB5 in 6.5% glucose + 6.5% xylose at pH 5.0 or pH 5.75 ± 0.4% acetate at 30°C

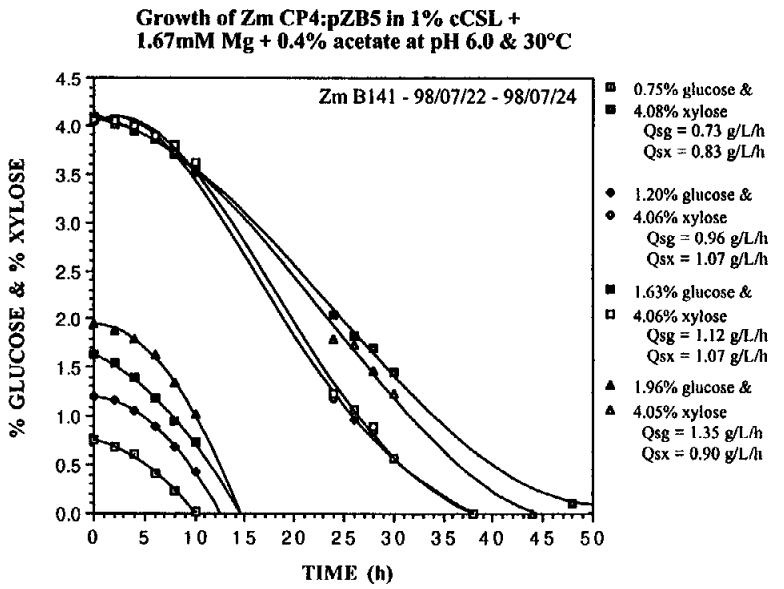


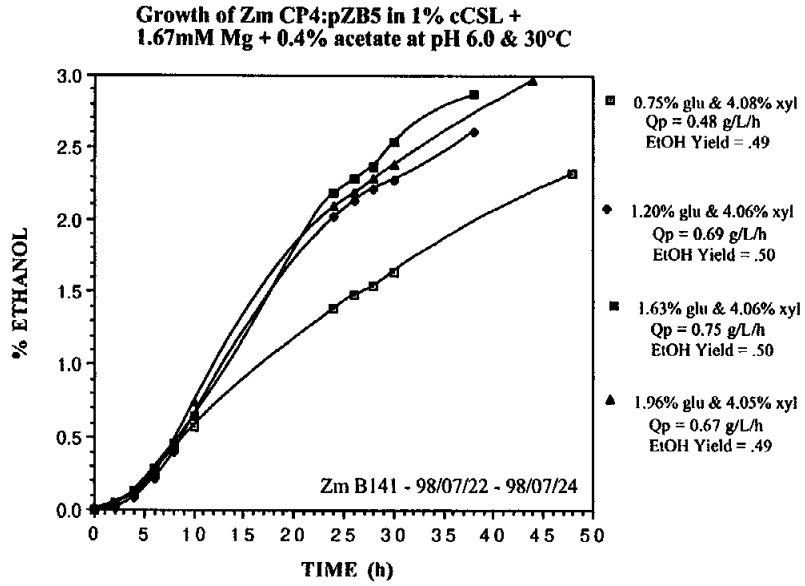

Zm B132 - 98/05/05 - 98/05/08
O.D. 600 nm.
TIME (h)
pH 5.0
6.61% glu & 6.60% xyl ZM
Biomass = 0.58 g/L/h
Y x/s = .017
pH 5.75
6.57% glu & 6.68% xyl ZM
Biomass = 1.57g/L/h
Y x/s = .020
pH 5.75 + 0.4% acetate
6.67% glu & 6.86% xyl ZM
Biomass = 1.16 g/L
Y x/s = .017

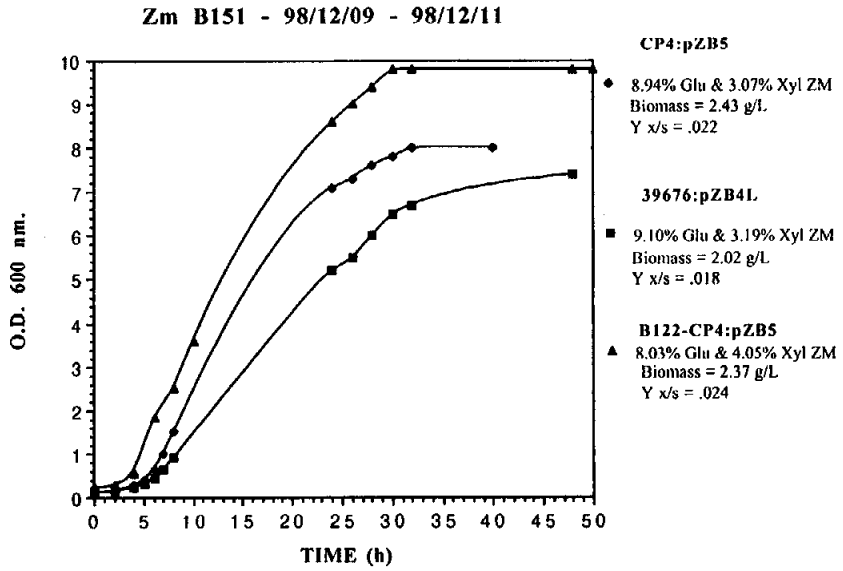

Growth of "adapted" ATCC 39676:pZB4L in 6.5% glucose
+ 6.5% xylose at pH 5.0 or pH 5.75 ± 0.4% acetate at 30°C
Zm B132 - 98/05/05 - 98/05/08
% GLUCOSE or % XYLOSE
TIME (h)
pH 5.0
6.61% glucose &
6.60% xylose ZM
Qsg = 0.53 g/L/h
Qsx = 0.13 g/L/h
pH 5.75
6.57% glucose &
6.68% xylose ZM
Qsg = 1.10 g/L/h
Qsx = 0.46 g/L/h
pH 5.75 + 0.4% acetate
6.67% glucose &
6.86% xylose ZM
Qsg = 0.76 g/L/h
Qsx = 0.56 g/L/h

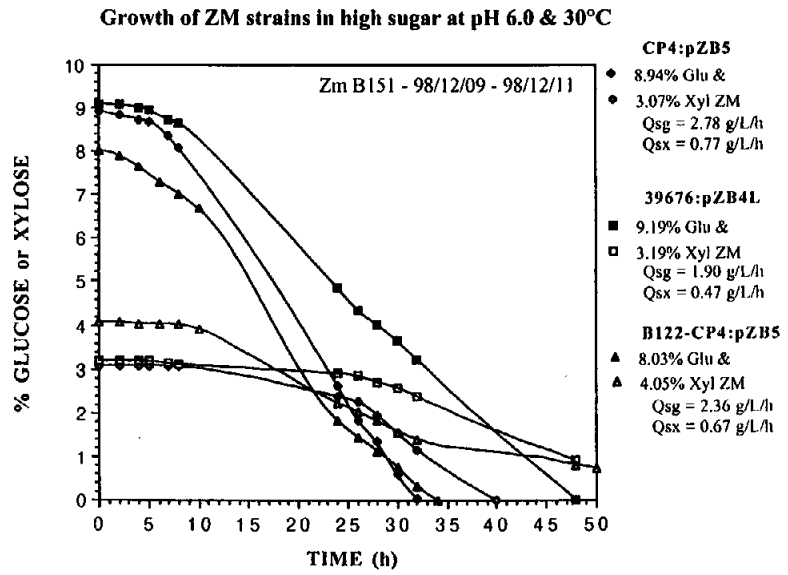

Growth of "adapted" ATCC 39676:pZB4L in 6.5% glucose
+ 6.5% xylose at pH 5.0 or pH 5.75 ± 0.4% acetate at 30°C
% ETHANOL
TIME (h)
Zm B132 - 98/05/05 - 98/05/08
pH 5.0
6.61% glu & 6.60% xyl ZM
Qp = 0.30 g/L/h
EtOH Yield = .46
pH 5.75
6.57% glu & 6.68% xyl ZM
Qp = 0.64 g/L/h
EtOH Yield = .47
pH 5.75 + 0.4% acetate
6.67% glu & 6.86% xyl ZM
Qp = 0.62 g/L/h
EtOH Yield = .47

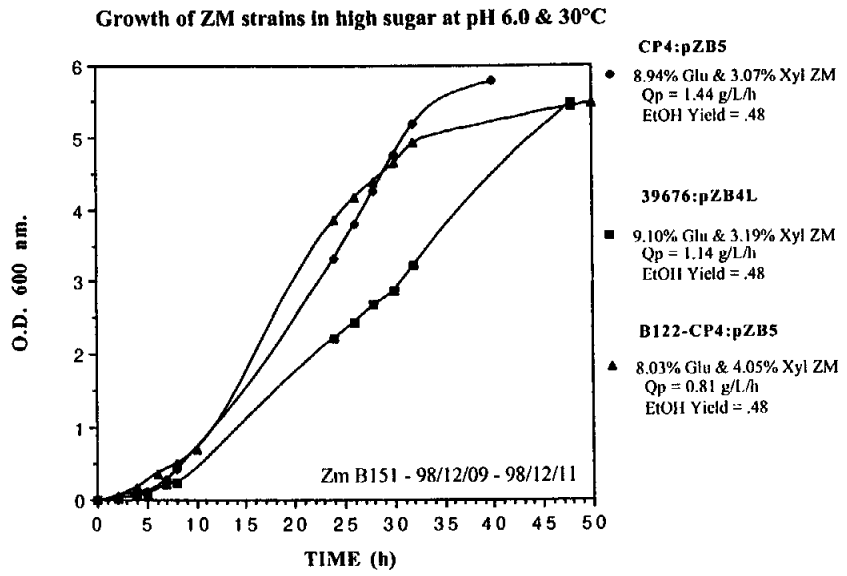

Zm B141 - 98/07/22 - 98/07/24
O.D. 600 nm.
TIME (h)
0.75% glu & 4.08% xyl
Biomass = 1.01 g/L
Y x/s = .030
1.20% glu & 4.06% xyl
Biomass = 1.30 g/L
Y x/s = .028
1.63% glu & 4.06% xyl
Biomass = 1.36 g/L
Y x/s = .029
1.96% glu & 4.05% xyl
Biomass = 1.26 g/L
Y x/s = .026


Growth of Zm CP4:pZB5 in 1% cCSL +
1.67mM Mg + 0.4% acetate at pH 6.0 & 30°C
Zm B141 - 98/07/22 - 98/07/24
% GLUCOSE & % XYLOSE
TIME (h)
0.75% glucose &
4.08% xylose
Qsg = 0.73 g/L/h
Qsx = 0.83 g/L/h
1.20% glucose &
4.06% xylose
Qsg = 0.96 g/L/h
Qsx = 1.07 g/L/h
1.63% glucose &
4.06% xylose
Qsg = 1.12 g/L/h
Qsx = 1.07 g/L/h
1.96% glucose &
4.05% xylose
Qsg = 1.35 g/L/h
Qsx = 0.90 g/L/h


Growth of Zm CP4:pZB5 in 1% cCSL +
1.67mM Mg + 0.4% acetate at pH 6.0 & 30°C
Zm B141 - 98/07/22 - 98/07/24
% ETHANOL
TIME (h)
0.75% glu & 4.08% xyl
Qp = 0.48 g/L/h
EtOH Yield = .49
1.20% glu & 4.06% xyl
Qp = 0.69 g/L/h
EtOH Yield = .50
1.63% glu & 4.06% xyl
Qp = 0.75 g/L/h
EtOH Yield = .50
1.96% glu & 4.05% xyl
Qp = 0.67 g/L/h
EtOH Yield = .49

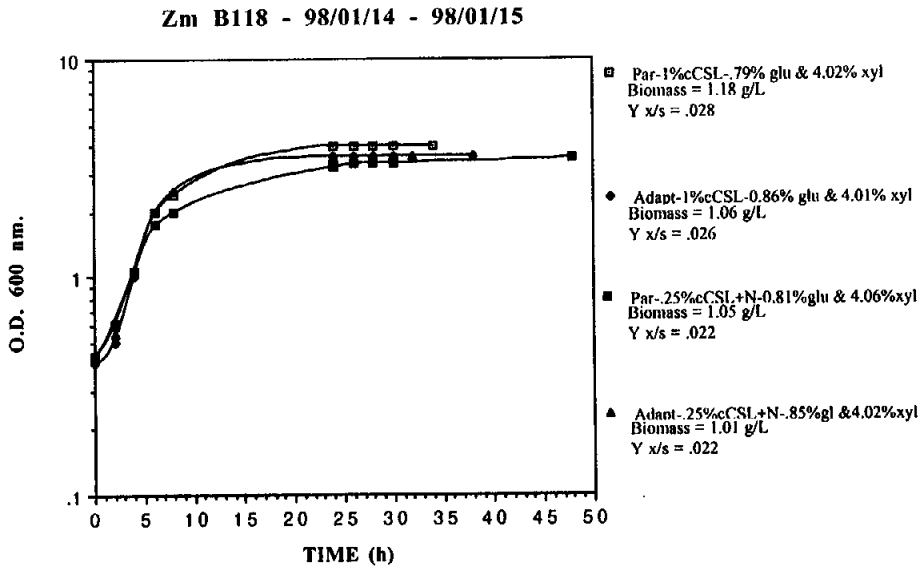

Zm B151 - 98/12/09 - 98/12/11
O.D. 600 nm.
TIME (h)
CP4:pZB5
8.94% Glu & 3.07% Xyl ZM
Biomass = 2.43 g/L
Y x/s = .022
39676:pZB4L
9.10% Glu & 3.19% Xyl ZM
Biomass = 2.02 g/L
Y x/s = .018
B122-CP4:pZB5
8.03% Glu & 4.05% Xyl ZM
Biomass = 2.37 g/L
Y x/s = .024

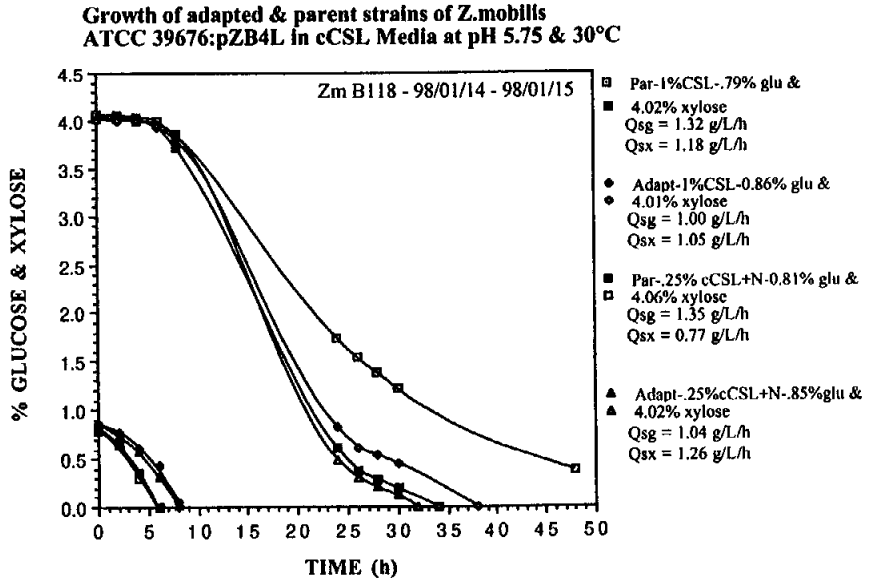

Growth of ZM strains in high sugar at pH 6.0 & 30°C
Zm B151 - 98/12/09 - 98/12/11
% GLUCOSE or XYLOSE
TIME (h)
CP4:pZB5
8.94% Glu &
3.07% Xyl ZM
Qsg = 2.78 g/L/h
Qsx = 0.77 g/L/h
39676:pZB4L
9.19% Glu &
3.19% Xyl ZM
Qsg = 1.90 g/L/h
Qsx = 0.47 g/L/h
B122-CP4:pZB5
8.03% Glu &
4.05% Xyl ZM
Qsg = 2.36 g/L/h
Qsx = 0.67 g/L/h

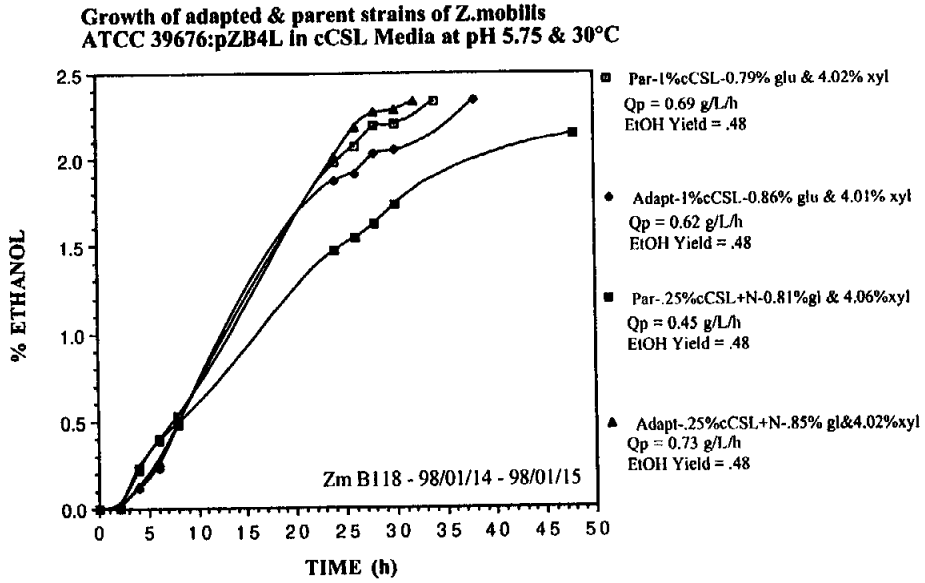

Growth of ZM strains in high sugar at pH 6.0 & 30°C
O.D. 600 nm.
TIME (h)
Zm B151 - 98/12/09 - 98/12/11
CP4:pZB5
8.94% Glu & 3.07% Xyl ZM
Qp = 1.44 g/L/h
EtOH Yield = .48
39676:pZB4L
9.10% Glu & 3.19% Xyl ZM
Qp = 1.14 g/L/h
EtOH Yield = .48
B122-CP4:pZB5
8.03% Glu & 4.05% Xyl ZM
Qp = 0.81 g/L/h
EtOH Yield = .48

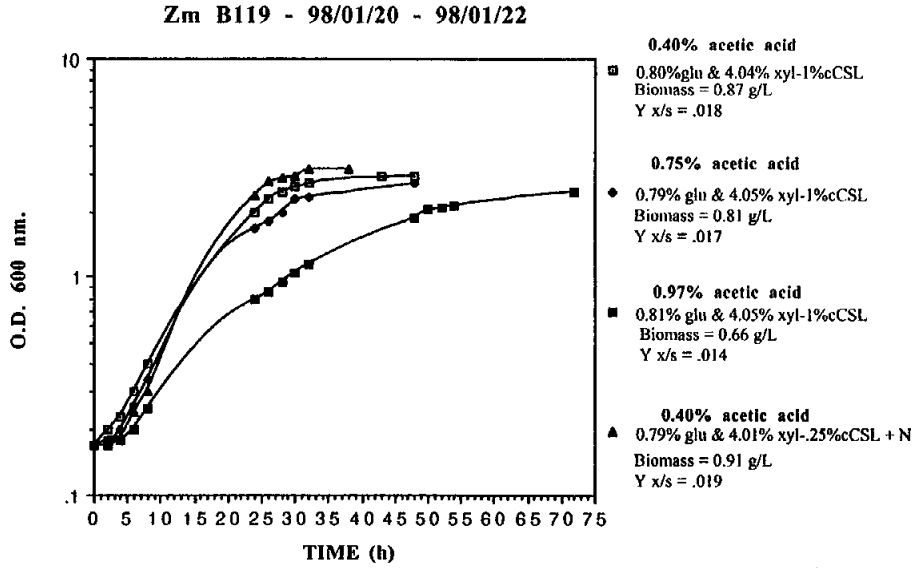
APPENDIX I

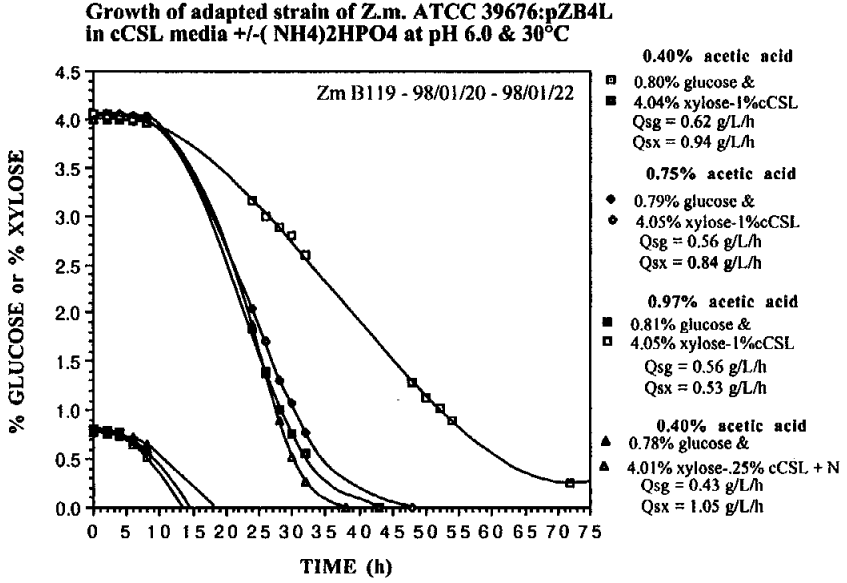
Graphical summaries for subcontract extension Task 4

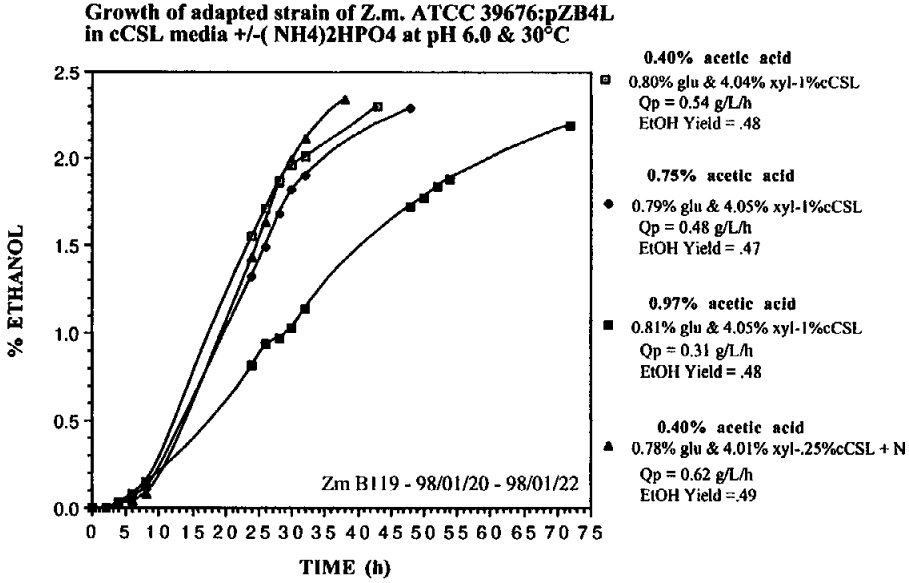

Zm B118 - 98/01/14 - 98/01/15
O.D. 600 nm.
TIME (h)
Par-1%cCSL-.79% glu & 4.02% xyl
Biomass = 1.18 g/L
Y x/s = .028
Adapt-1%cCSL-0.86% glu & 4.01% xyl
Biomass = 1.06 g/L
Y x/s = .026
Par-.25%cCSL+N-0.81%glu & 4.06%xyl
Biomass = 1.05 g/L
Y x/s = .022
Adapt-.25%cCSL+N-.85%gl &4.02%xyl
Biomass = 1.01 g/L
Y x/s = .022

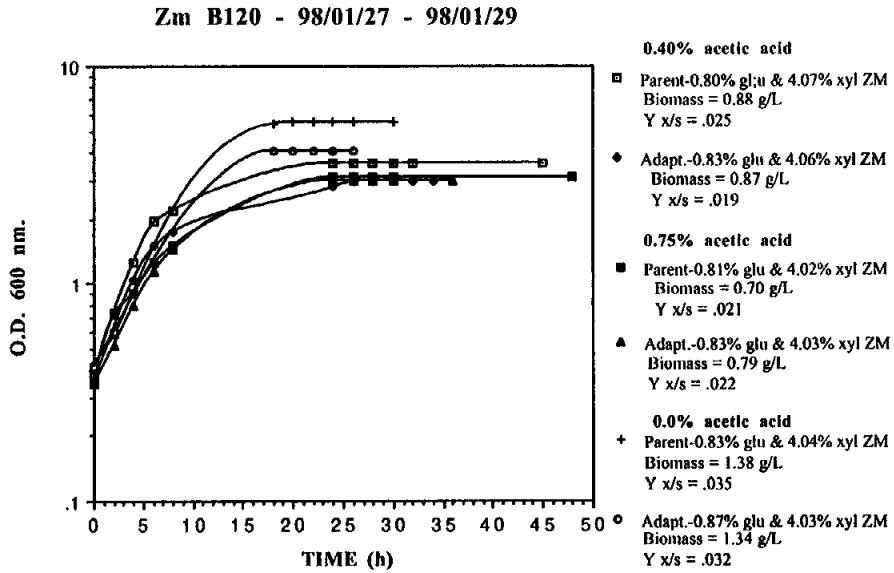

Growth of adapted & parent strains of Z.mobilis
ATCC 39676:pZB4L in cCSL Media at pH 5.75 & 30°C
Zm B118 - 98/01/14 - 98/01/15
% GLUCOSE & XYLOSE
TIME (h)
Par-1%CSL-.79% glu &
4.02% xylose
Qsg = 1.32 g/L/h
Qsx = 1.18 g/L/h
Adapt-1%CSL-0.86% glu &
4.01% xylose
Qsg = 1.00 g/L/h
Qsx = 1.05 g/L/h
Par-.25% cCSL+N-0.81% glu &
4.06% xylose
Qsg = 1.35 g/L/h
Qsx = 0.77 g/L/h
Adapt-.25%cCSL+N-.85%glu &
4.02% xylose
Qsg = 1.04 g/L/h
Qsx = 1.26 g/L/h

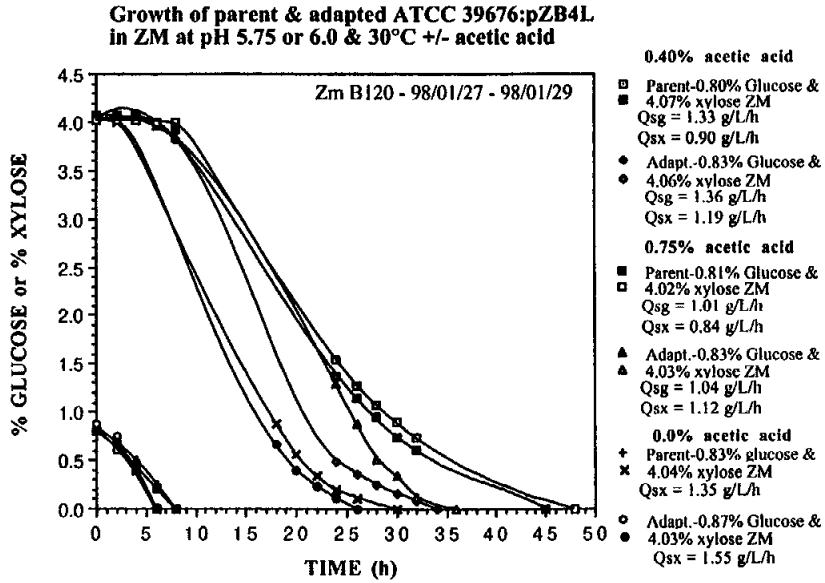

Growth of adapted & parent strains of Z.mobilis
ATCC 39676:pZB4L in cCSL Media at pH 5.75 & 30°C
% ETHANOL
TIME (h)
Zm B118 - 98/01/14 - 98/01/15
Par-1%cCSL-0.79% glu & 4.02% xyl
Qp = 0.69 g/L/h
EtOH Yield = .48
Adapt-1%cCSL-0.86% glu & 4.01% xyl
Qp = 0.62 g/L/h
EtOH Yield = .48
Par-.25%cCSL+N-0.81%gl & 4.06%xyl
Qp = 0.45 g/L/h
EtOH Yield = .48
Adapt-.25%cCSL+N-.85% gl&4.02%xyl
Qp = 0.73 g/L/h
EtOH Yield = .48

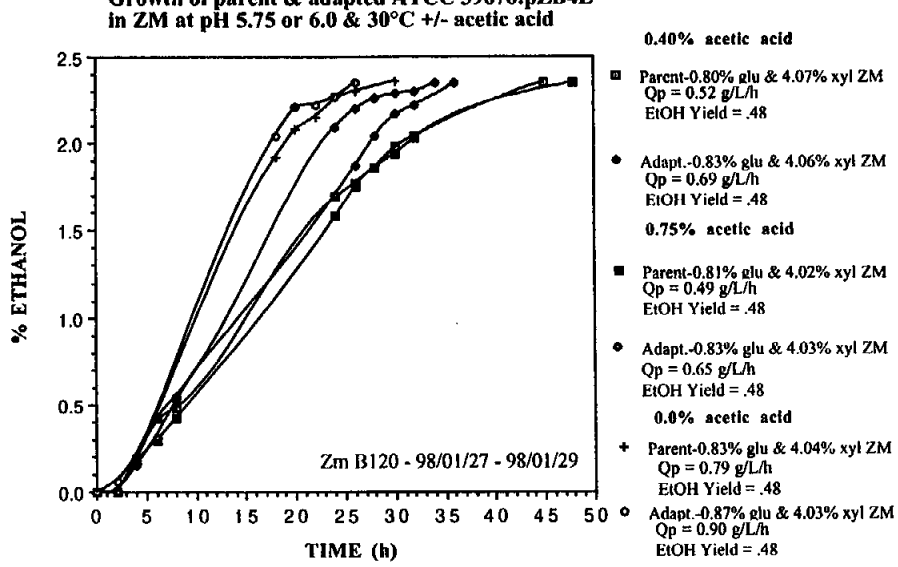

Zm B119 - 98/01/20 - 98/01/22
O.D. 600 nm.
TIME (h)
0.40% acetic acid
0.80%glu & 4.04% xyl-1%cCSL
Biomass = 0.87 g/L
Y x/s = .018
0.75% acetic acid
0.79% glu & 4.05% xyl-1%cCSL
Biomass = 0.81 g/L
Y x/s = .017
0.97% acetic acid
0.81% glu & 4.05% xyl-1%cCSL
Biomass = 0.66 g/L
Y x/s = .014
0.40% acetic acid
0.79% glu & 4.01% xyl-.25%cCSL + N
Biomass = 0.91 g/L
Y x/s = .019


Growth of adapted strain of Z.m. ATCC 39676:pZB4L
in cCSL media +/-(NH4)2HPO4 at pH 6.0 & 30°C
Zm B119 - 98/01/20 - 98/01/22
% GLUCOSE or % XYLOSE
TIME (h)
0.40% acetic acid
0.80% glucose &
4.04% xylose-1%cCSL
Qsg = 0.62 g/L/h
Qsx = 0.94 g/L/h
0.75% acetic acid
0.79% glucose &
4.05% xylose-1%cCSL
Qsg = 0.56 g/L/h
Qsx = 0.84 g/L/h
0.97% acetic acid
0.81% glucose &
4.05% xylose-1%cCSL
Qsg = 0.56 g/L/h
Qsx = 0.53 g/L/h
0.40% acetic acid
0.78% glucose &
4.01% xylose-.25% cCSL + N
Qsg = 0.43 g/L/h
Qsx = 1.05 g/L/h

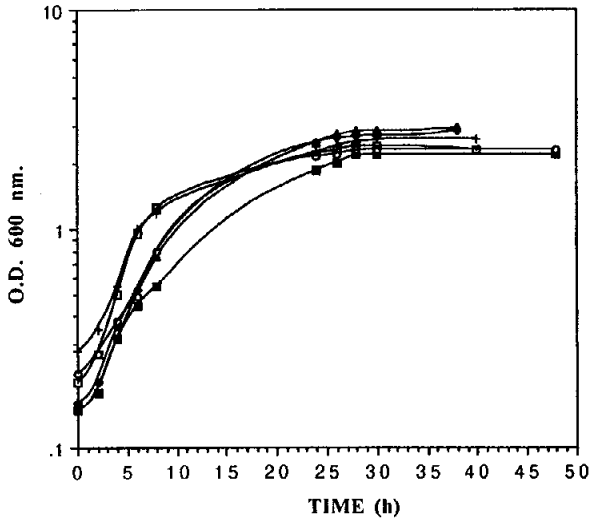

Growth of adapted strain of Z.m. ATCC 39676:pZB4L
in cCSL media +/-(NH4)2HPO4 at pH 6.0 & 30°C
Zm B119 - 98/01/20 - 98/01/22
% ETHANOL
TIME (h)
0.40% acetic acid
0.80% glu & 4.04% xyl-1%cCSL
Qp = 0.54 g/L/h
EtOH Yield = .48
0.75% acetic acid
0.79% glu & 4.05% xyl-1%cCSL
Qp = 0.48 g/L/h
EtOH Yield = .47
0.97% acetic acid
0.81% glu & 4.05% xyl-1%cCSL
Qp = 0.31 g/L/h
EtOH Yield = .48
0.40% acetic acid
0.78% glu & 4.01% xyl-.25%cCSL + N
Qp = 0.62 g/L/h
EtOH Yield =.49


Zm B120 - 98/01/27 - 98/01/29
O.D. 600 nm.
TIME (h)
0.40% acetic acid
Parent-0.80% glu & 4.07% xyl ZM
Biomass = 0.88 g/L
Y x/s = .025
Adapt.-0.83% glu & 4.06% xyl ZM
Biomass = 0.87 g/L
Y x/s = .019
0.75% acetic acid
Parent-0.81% glu & 4.02% xyl ZM
Biomass = 0.70 g/L
Y x/s = .021
Adapt.-0.83% glu & 4.03% xyl ZM
Biomass = 0.79 g/L
Y x/s = .022
0.0% acetic acid
Parent-0.83% glu & 4.04% xyl ZM
Biomass = 1.38 g/L
Y x/s = .035
Adapt.-0.87% glu & 4.03% xyl ZM
Biomass = 1.34 g/L
Y x/s = .032


Growth of parent & adapted ATCC 39676:pZB4L
in ZM at pH 5.75 or 6.0 & 30°C +/- acetic acid
Zm B120 - 98/01/27 - 98/01/29
% GLUCOSE or % XYLOSE
TIME (h)
0.40% acetic acid
Parent-0.80% Glucose & 4.07% xylose ZM
Qsg = 1.33 g/L/h
Qsx = 0.90 g/L/h
Adapt.-0.83% Glucose & 4.06% xylose ZM
Qsg = 1.36 g/L/h
Qsx = 1.19 g/L/h
0.75% acetic acid
Parent-0.81% Glucose & 4.02% xylose ZM
Qsg = 1.01 g/L/h
Qsx = 0.84 g/L/h
Adapt.-0.83% Glucose & 4.03% xylose ZM
Qsg = 1.04 g/L/h
Qsx = 1.12 g/L/h
0.0% acetic acid
Parent-0.83% glucose & 4.04% xylose ZM
Qsx = 1.35 g/L/h
Adapt.-0.87% Glucose & 4.03% xylose ZM
Qsx = 1.55 g/L/h

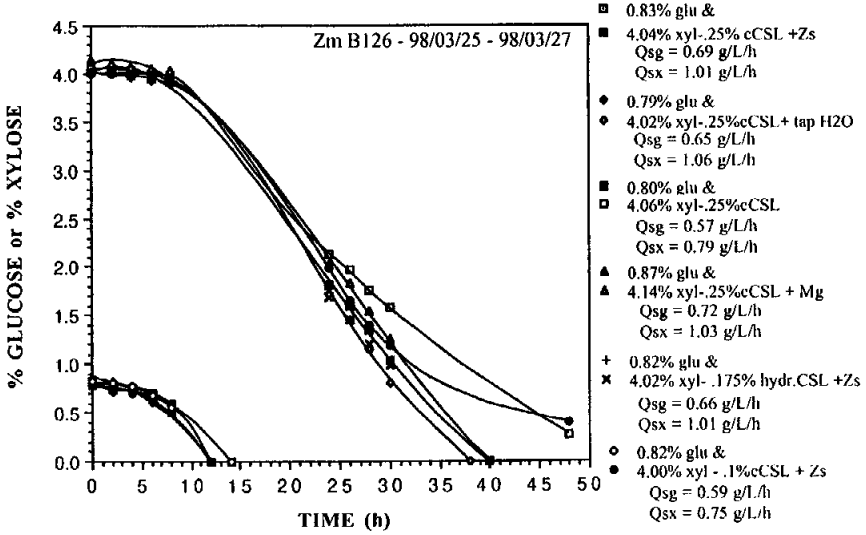

Growth of parent & adapted ATCC 39676:pZB4L
in ZM at pH 5.75 or 6.0 & 30°C +/- acetic acid
Zm B120 - 98/01/27 - 98/01/29
% ETHANOL
TIME (h)
0.40% acetic acid
Parent-0.80% glu & 4.07% xyl ZM
Qp = 0.52 g/L/h
EtOH Yield = .48
Adapt.-0.83% glu & 4.06% xyl ZM
Qp = 0.69 g/L/h
EtOH Yield = .48
0.75% acetic acid
Parent-0.81% glu & 4.02% xyl ZM
Qp = 0.49 g/L/h
EtOH Yield = .48
Adapt.-0.83% glu & 4.03% xyl ZM
Qp = 0.65 g/L/h
EtOH Yield = .48
0.0% acetic acid
Parent-0.83% glu & 4.04% xyl ZM
Qp = 0.79 g/L/h
EtOH Yield = .48
Adapt.-0.87% glu & 4.03% xyl ZM
Qp = 0.90 g/L/h
EtOH Yield = .48

Zm B126 - 98/03/25 - 98/03/27



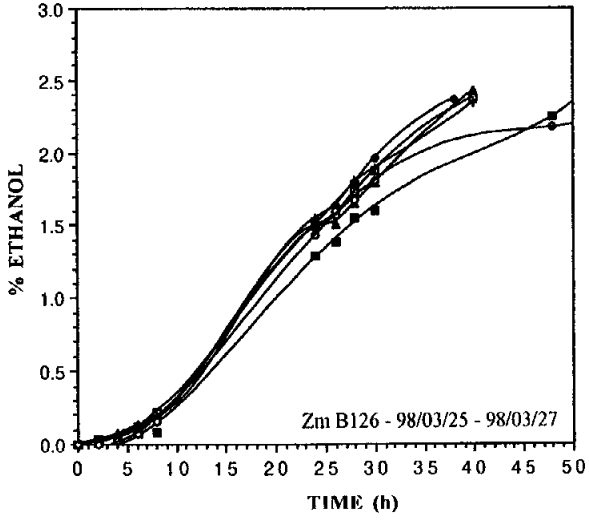
- 0.83% glu & 4.04% xyl-.25%cCSL+Zs
  Biomass = 0.72g/L
  Y x/s = .020
- 0.79%gl & 4.02%xy-.25%cCSL+Tap H2O
  Biomass = 0.87 g/L
  Y x/s = .022
- 0.80%gln & 4.06% xyl-.25%cCSL
  Biomass = 0.65g/L
  Y x/s = .021
- 0.875glu & 4.14%xyl-.25%cCSL+Mg
  Biomass = 0.82 g/L
  Y x/s = .022
- 0.79%gl & 4.02%xy-.18%hydr.CSL +Zs
  Biomass = 0.77g/L
  Y x/s = .020
- 0.82%glu & 4.00%xyl-.1%cCSL+Zs
  Biomass = 0.68 g/L
  Y x/s = .020

Growth of "adapted" ATCC 39676:pZB4L in cCSL Media

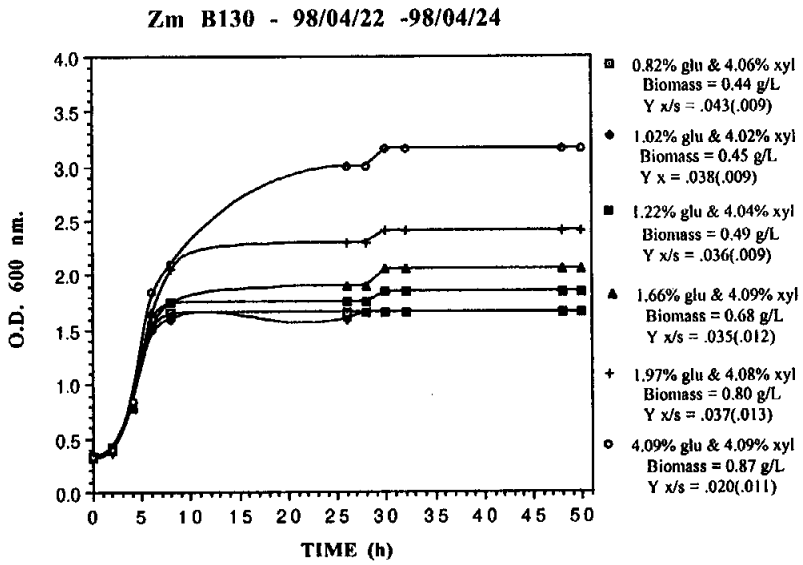


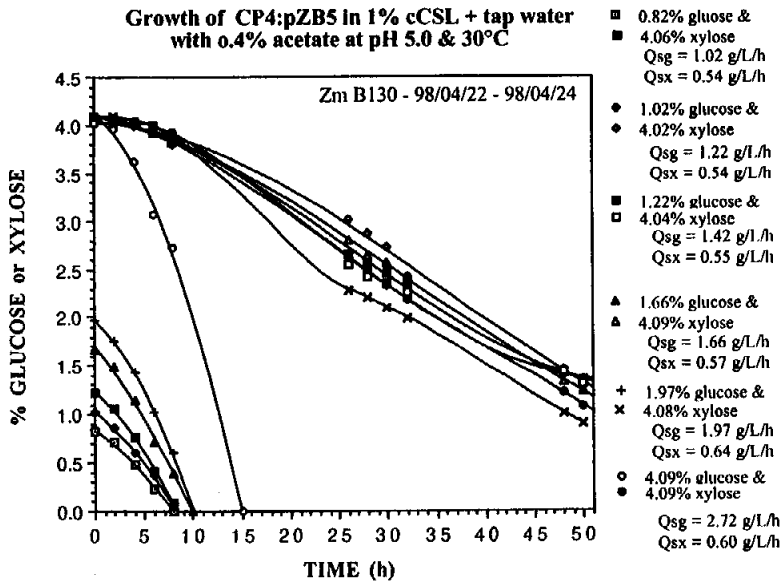
- 0.83% glu &
  4.04% xyl-.25% cCSL +Zs
  Qsg = 0.69 g/L/h
  Qsx = 1.01 g/L/h
- 0.79% glu &
  4.02% xyl-.25%cCSL+ tap H2O
  Qsg = 0.65 g/L/h
  Qsx = 1.06 g/L/h
- 0.80% glu &
  4.06% xyl-.25%cCSL
  Qsg = 0.57 g/L/h
  Qsx = 0.79 g/L/h
- 0.87% glu &
  4.14% xyl-.25%cCSL + Mg
  Qsg = 0.72 g/L/h
  Qsx = 1.03 g/L/h
- 0.82% glu &
  4.02% xyl- .175% hydr.CSL +Zs
  Qsg = 0.66 g/L/h
  Qsx = 1.01 g/L/h
- 0.82% glu &
  4.00% xyl - .1%cCSL + Zs
  Qsg = 0.59 g/L/h
  Qsx = 0.75 g/L/h

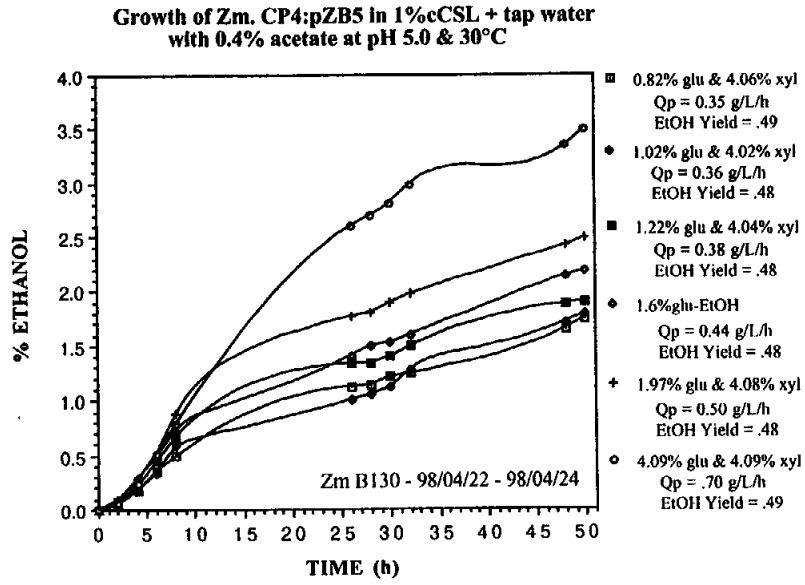
Growth of "adapted" ATCC 39676:pZB4L in cCSL Media

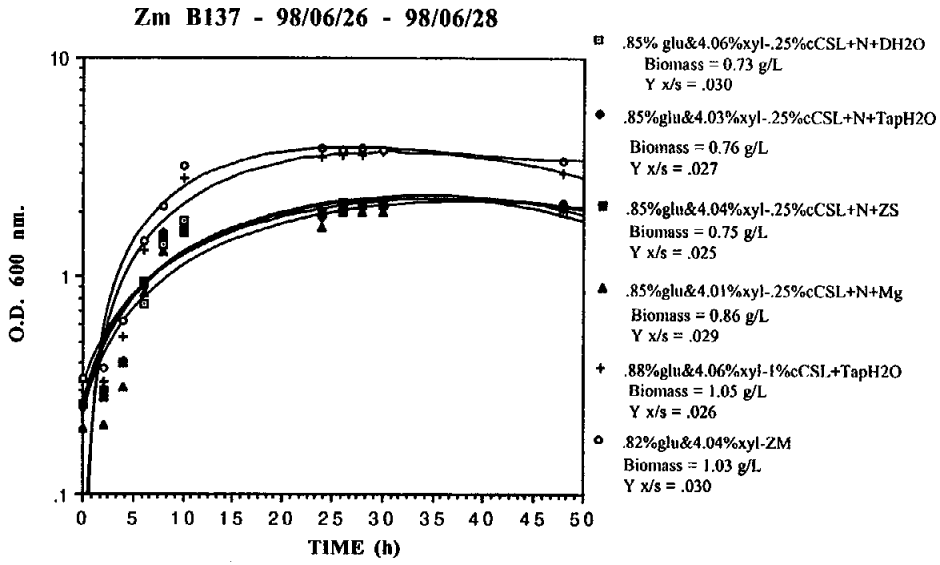


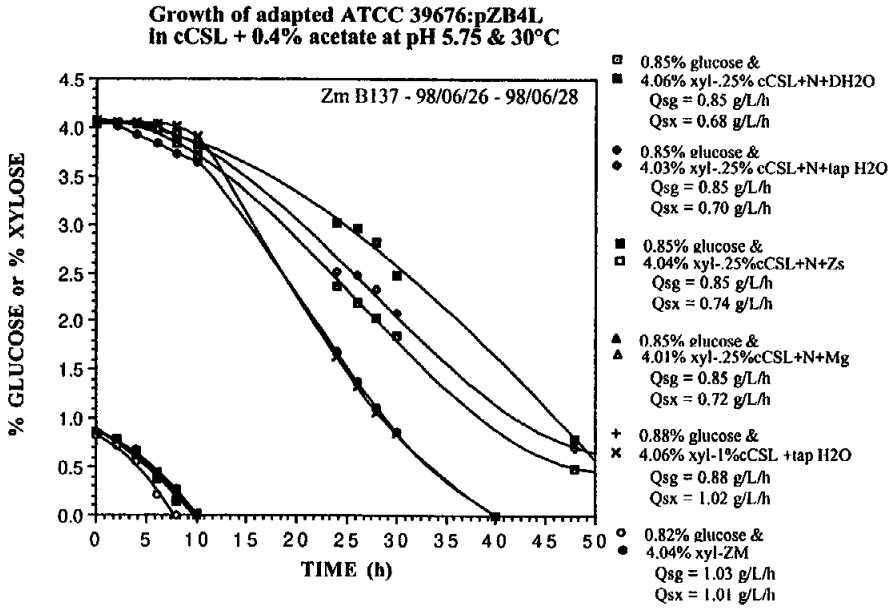
- 0.83%glu & 4.04%xyl-.25%cCSL +Zs
  Qp = 0.60 g/L/h
  EtOH Yield = .49
- 0.79%gl & 4.02%xy-.25%cCSL+Tap H20
  Qp = 0.62 g/L/h
  EtOH Yield = .49
- 0.80%glu & 4.06% xyl-.25%cCSL
  Qp = 0.47 g/L/h
  EtOH Yield = .49
- 0.87%glu & 4.14%xyl-.25%cCSL+Mg
  Qp = 0.61 g/L/h
  EtOH Yield = .49
- 0.79%glu &4.02%xyl-.18%hydr.CSL+Zs
  Qp = 0.59 g/L/h
  EtOH Yield = .49
- 0.82%glu & 4.00%xyl-.1%cCSL+Zs
  Qp = 0.45 g/L/h
  EtOH Yield = .48

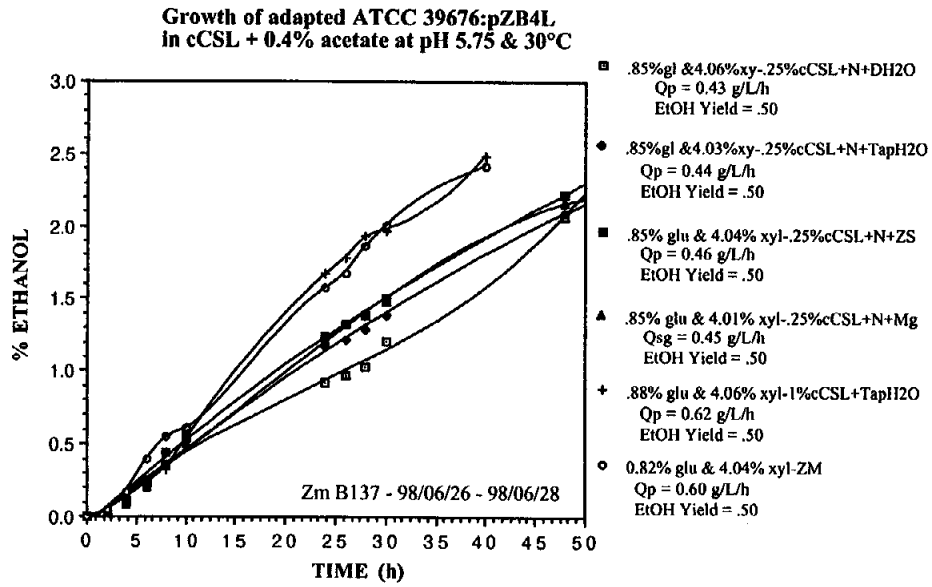

Zm B130 - 98/04/22 -98/04/24
O.D. 600 nm.
TIME (h)
0.82% glu & 4.06% xyl
Biomass = 0.44 g/L
Y x/s = .043(.009)
1.02% glu & 4.02% xyl
Biomass = 0.45 g/L
Y x = .038(.009)
1.22% glu & 4.04% xyl
Biomass = 0.49 g/L
Y x/s = .036(.009)
1.66% glu & 4.09% xyl
Biomass = 0.68 g/L
Y x/s = .035(.012)
1.97% glu & 4.08% xyl
Biomass = 0.80 g/L
Y x/s = .037(.013)
4.09% glu & 4.09% xyl
Biomass = 0.87 g/L
Y x/s = .020(.011)

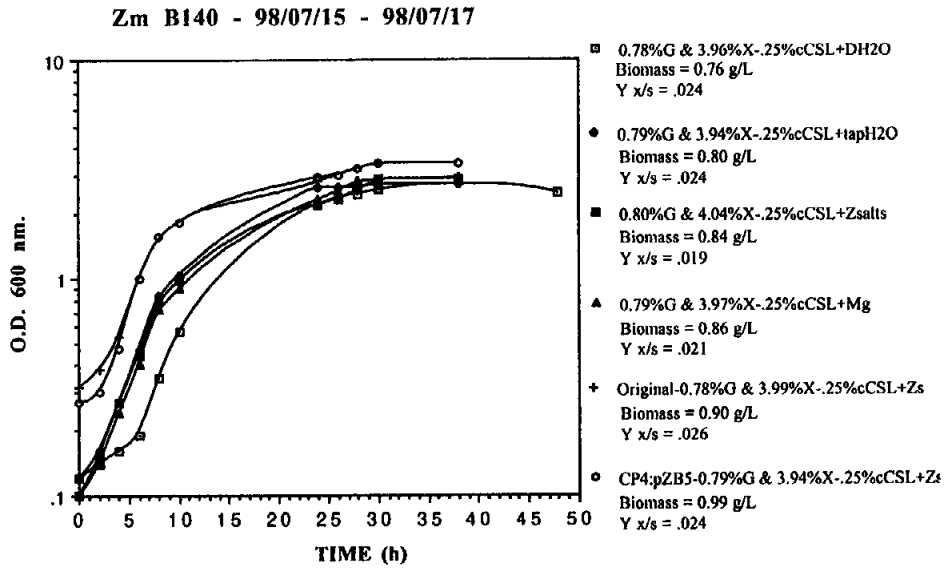

Growth of CP4:pZB5 in 1% cCSL + tap water
with 0.4% acetate at pH 5.0 & 30°C
Zm B130 - 98/04/22 - 98/04/24
% GLUCOSE or XYLOSE
TIME (h)
0.82% gluose &
4.06% xylose
Qsg = 1.02 g/L/h
Qsx = 0.54 g/L/h
1.02% glucose &
4.02% xylose
Qsg = 1.22 g/L/h
Qsx = 0.54 g/L/h
1.22% glucose &
4.04% xylose
Qsg = 1.42 g/L/h
Qsx = 0.55 g/L/h
1.66% glucose &
4.09% xylose
Qsg = 1.66 g/L/h
Qsx = 0.57 g/L/h
1.97% glucose &
4.08% xylose
Qsg = 1.97 g/L/h
Qsx = 0.64 g/L/h
4.09% glucose &
4.09% xylose
Qsg = 2.72 g/L/h
Qsx = 0.60 g/L/h

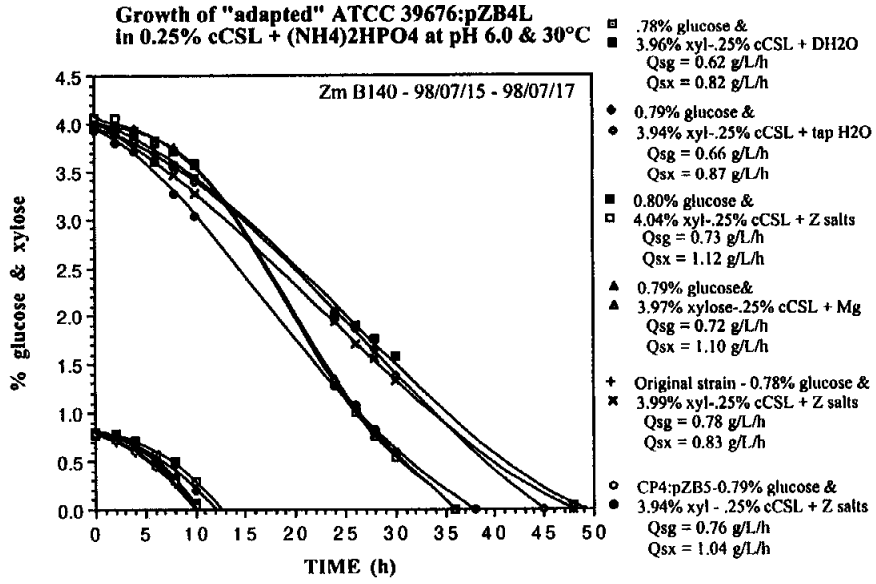

Growth of Zm. CP4:pZB5 in 1%cCSL + tap water
with 0.4% acetate at pH 5.0 & 30°C
Zm B130 - 98/04/22 - 98/04/24
% ETHANOL
TIME (h)
0.82% glu & 4.06% xyl
Qp = 0.35 g/L/h
EtOH Yield = .49
1.02% glu & 4.02% xyl
Qp = 0.36 g/L/h
EtOH Yield = .48
1.22% glu & 4.04% xyl
Qp = 0.38 g/L/h
EtOH Yield = .48
1.6%glu-EtOH
Qp = 0.44 g/L/h
EtOH Yield = .48
1.97% glu & 4.08% xyl
Qp = 0.50 g/L/h
EtOH Yield = .48
4.09% glu & 4.09% xyl
Qp = .70 g/L/h
EtOH Yield = .49

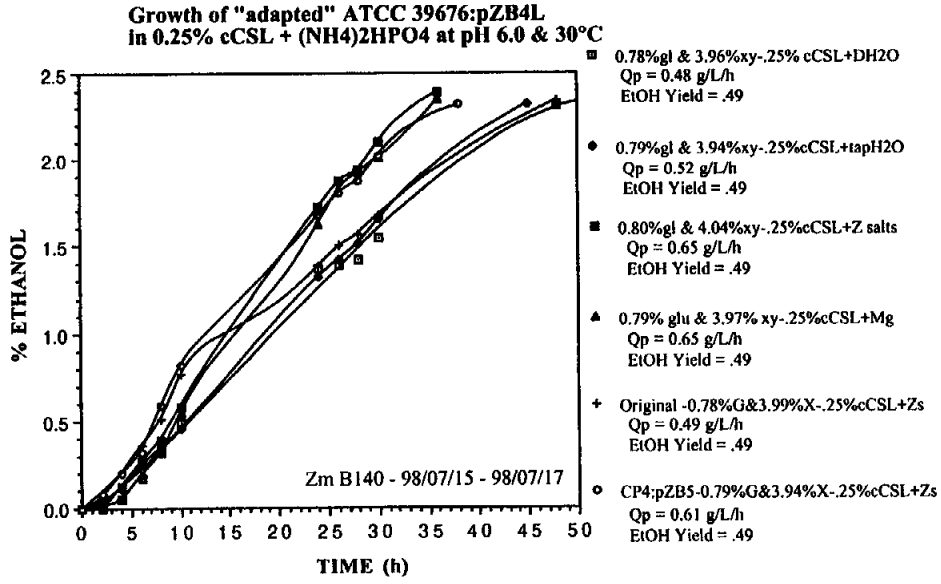

Zm B137 - 98/06/26 - 98/06/28
O.D. 600 nm.
TIME (h)
.85% glu&4.06%xyl-.25%cCSL+N+DH2O
Biomass = 0.73 g/L
Y x/s = .030
.85%glu&4.03%xyl-.25%cCSL+N+TapH2O
Biomass = 0.76 g/L
Y x/s = .027
.85%glu&4.04%xyl-.25%cCSL+N+ZS
Biomass = 0.75 g/L
Y x/s = .025
.85%glu&4.01%xyl-.25%cCSL+N+Mg
Biomass = 0.86 g/L
Y x/s = .029
.88%glu&4.06%xyl-1%cCSL+TapH2O
Biomass = 1.05 g/L
Y x/s = .026
.82%glu&4.04%xyl-ZM
Biomass = 1.03 g/L
Y x/s = .030


Growth of adapted ATCC 39676:pZB4L
in cCSL + 0.4% acetate at pH 5.75 & 30°C
Zm B137 - 98/06/26 - 98/06/28
% GLUCOSE or % XYLOSE
TIME (h)
0.85% glucose &
4.06% xyl-.25% cCSL+N+DH2O
Qsg = 0.85 g/L/h
Qsx = 0.68 g/L/h
0.85% glucose &
4.03% xyl-.25% cCSL+N+tap H2O
Qsg = 0.85 g/L/h
Qsx = 0.70 g/L/h
0.85% glucose &
4.04% xyl-.25%cCSL+N+Zs
Qsg = 0.85 g/L/h
Qsx = 0.74 g/L/h
0.85% glucose &
4.01% xyl-.25%cCSL+N+Mg
Qsg = 0.85 g/L/h
Qsx = 0.72 g/L/h
0.88% glucose &
4.06% xyl-1%cCSL +tap H2O
Qsg = 0.88 g/L/h
Qsx = 1.02 g/L/h
0.82% glucose &
4.04% xyl-ZM
Qsg = 1.03 g/L/h
Qsx = 1.01 g/L/h


Growth of adapted ATCC 39676:pZB4L
in cCSL + 0.4% acetate at pH 5.75 & 30°C
Zm B137 - 98/06/26 - 98/06/28
% ETHANOL
TIME (h)
.85%gl &4.06%xy-.25%cCSL+N+DH2O
Qp = 0.43 g/L/h
EtOH Yield = .50
.85%gl &4.03%xy-.25%cCSL+N+TapH2O
Qp = 0.44 g/L/h
EtOH Yield = .50
.85% glu & 4.04% xyl-.25%cCSL+N+ZS
Qp = 0.46 g/L/h
EtOH Yield = .50
.85% glu & 4.01% xyl-.25%cCSL+N+Mg
Qsg = 0.45 g/L/h
EtOH Yield = .50
.88% glu & 4.06% xyl-1%cCSL+TapH2O
Qp = 0.62 g/L/h
EtOH Yield = .50
0.82% glu & 4.04% xyl-ZM
Qp = 0.60 g/L/h
EtOH Yield = .50


Zm B140 - 98/07/15 - 98/07/17
O.D. 600 nm.
10
1
.1
0 5 10 15 20 25 30 35 40 45 50
TIME (h)
0.78%G & 3.96%X-.25%cCSL+DH2O
Biomass = 0.76 g/L
Y x/s = .024
0.79%G & 3.94%X-.25%cCSL+tapH2O
Biomass = 0.80 g/L
Y x/s = .024
0.80%G & 4.04%X-.25%cCSL+Zsalts
Biomass = 0.84 g/L
Y x/s = .019
0.79%G & 3.97%X-.25%cCSL+Mg
Biomass = 0.86 g/L
Y x/s = .021
Original-0.78%G & 3.99%X-.25%cCSL+Zs
Biomass = 0.90 g/L
Y x/s = .026
CP4:pZB5-0.79%G & 3.94%X-.25%cCSL+Zs
Biomass = 0.99 g/L
Y x/s = .024


Growth of "adapted" ATCC 39676:pZB4L
in 0.25% cCSL + (NH4)2HPO4 at pH 6.0 & 30°C
Zm B140 - 98/07/15 - 98/07/17
% glucose & xylose
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
0 5 10 15 20 25 30 35 40 45 50
TIME (h)
.78% glucose &
3.96% xyl-.25% cCSL + DH2O
Qsg = 0.62 g/L/h
Qsx = 0.82 g/L/h
0.79% glucose &
3.94% xyl-.25% cCSL + tap H2O
Qsg = 0.66 g/L/h
Qsx = 0.87 g/L/h
0.80% glucose &
4.04% xyl-.25% cCSL + Z salts
Qsg = 0.73 g/L/h
Qsx = 1.12 g/L/h
0.79% glucose&
3.97% xylose-.25% cCSL + Mg
Qsg = 0.72 g/L/h
Qsx = 1.10 g/L/h
Original strain - 0.78% glucose &
3.99% xyl-.25% cCSL + Z salts
Qsg = 0.78 g/L/h
Qsx = 0.83 g/L/h
CP4:pZB5-0.79% glucose &
3.94% xyl - .25% cCSL + Z salts
Qsg = 0.76 g/L/h
Qsx = 1.04 g/L/h


Growth of "adapted" ATCC 39676:pZB4L
in 0.25% cCSL + (NH4)2HPO4 at pH 6.0 & 30°C
% ETHANOL
2.5
2.0
1.5
1.0
0.5
0.0
Zm B140 - 98/07/15 - 98/07/17
0 5 10 15 20 25 30 35 40 45 50
TIME (h)
0.78%gl & 3.96%xy-.25% cCSL+DH2O
Qp = 0.48 g/L/h
EtOH Yield = .49
0.79%gl & 3.94%xy-.25%cCSL+tapH2O
Qp = 0.52 g/L/h
EtOH Yield = .49
0.80%gl & 4.04%xy-.25%cCSL+Z salts
Qp = 0.65 g/L/h
EtOH Yield = .49
0.79% glu & 3.97% xy-.25%cCSL+Mg
Qp = 0.65 g/L/h
EtOH Yield = .49
Original -0.78%G&3.99%X-.25%cCSL+Zs
Qp = 0.49 g/L/h
EtOH Yield = .49
CP4:pZB5-0.79%G&3.94%X-.25%cCSL+Zs
Qp = 0.61 g/L/h
EtOH Yield = .49

APPENDIX J

NREL Site Visit Seminar

March 2, 1998

"Performance Testing of Recombinant *Zymomonas*"

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NREL PROTECTED INFORMATION

March 2, 1998

Subject: Subcontract No. **ACO-8-17095-01**
NREL Site Visit Seminar - March 2, 1998

This document contains the color overhead projector transparencies that were used as part of the presentation by Professor Hugh Lawford at the time of his visit to NREL March 2-3, 1998.

The subject of the presentation is captured in the title: *Performance Testing of Recombinant Zymomonas* - this comparative physiological assessment focused on pH-stat batch fermentations and three (3) recombinant strains of *Z. mobilis*.

Performance Testing of Recombinant *Zymomonas*

Hugh Lawford
Bio-engineering Laboratory
Department of Biochemistry
UNIVERSITY OF TORONTO
Toronto, Ontario, CANADA

National Renewable Energy Laboratory
Biotechnology Centre for Fuels & Chemicals
Golden, Colorado, U.S.A.

March 2, 1998

BIOCATALYSTS

STRAINS TESTED.....

Phase I rec *Zymomonas mobilis* CP4:pZB5 ▲

Phase II rec *Zymomonas mobilis* 39676:pZB4L ○

Phase III "adapted" variant of rZm 39676:pZB4L(A) ●

Zymomonas media formulations

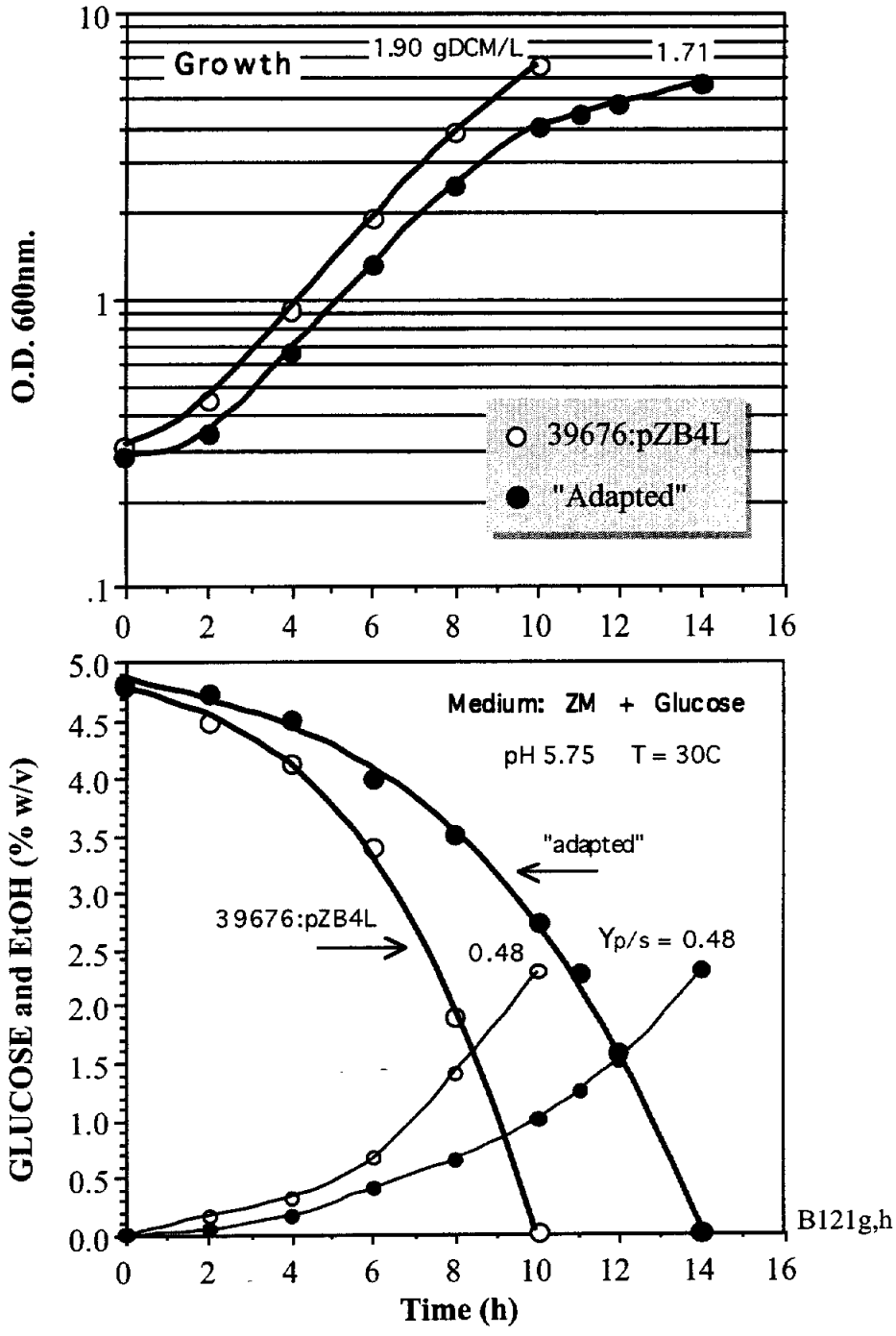
Ingredient (g)	Medium Designation				
			This work		
	RM[•]	ZM1[*]	ZM	cCSL	Lo cCSL
Yeast Extract (Difco)	10.0	3.0	5.0		
cCSL (mL)				50	12.5
NH_4Cl	-	0.8	0.8	-	-
$(NH_4)_2HPO_4$ (DAP)	-	-	-	-	1.23
KH_2PO_4	2.0	3.48	3.48	3.48	3.48
$MgSO_4.7H_2O$	-	1.0	1.0	1.0	1.0
$FeSO_4.7H_2O$	-	0.01	0.01	0.01	0.01
Citric acid	-	0.21	0.21	0.21	0.21
Distilled water (L)	1	1	1	1	1

Clarified CSL (cCSL) = CSL diluted 1:4, centrifuged and filter sterilzed

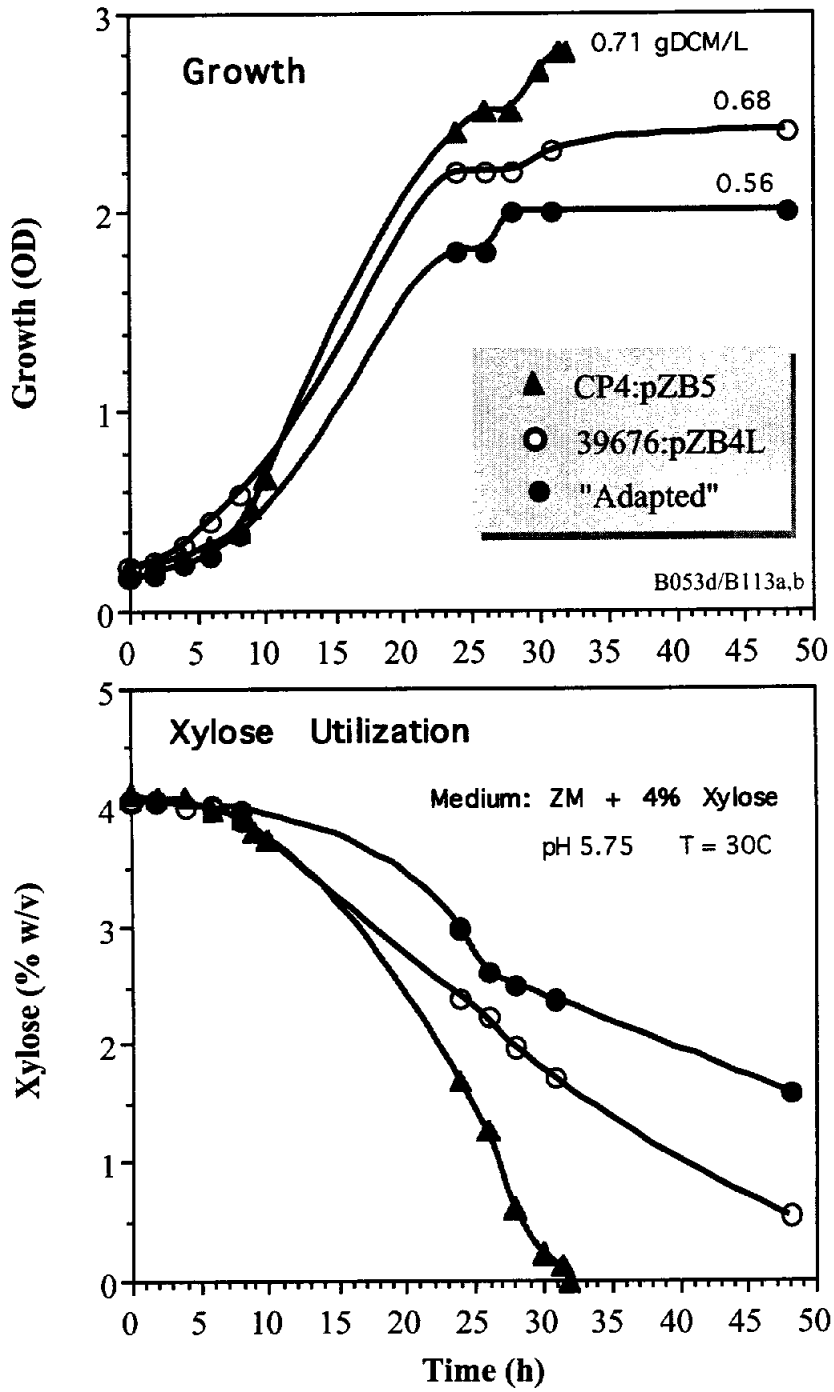
[•] Goodman *et al.* (1982) *Appl. Environ. Microbiol.*, 44(2): 496-498

[*] Lawford (1988) *Appl. Biochem. Biotechnol.*, 17: 203-209

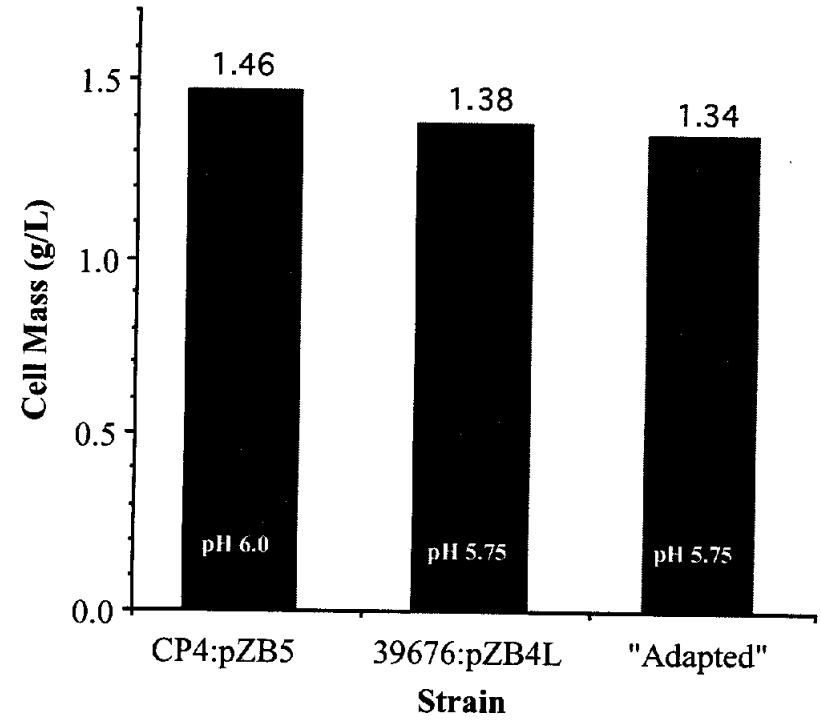
Comparative Growth & Fermentation Performance with Glucose as Sole Sugar

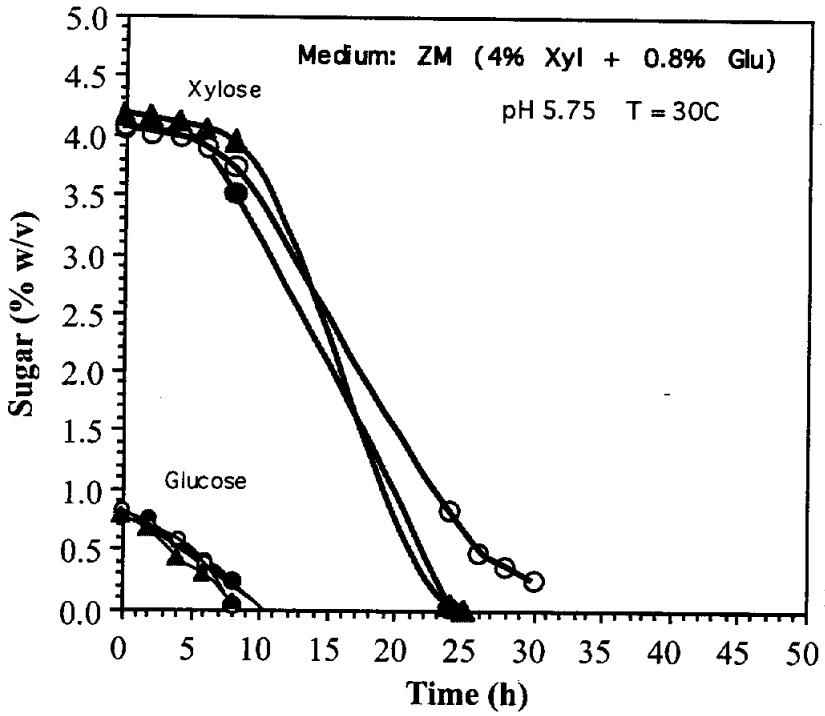


Comparative Growth & Fermentation Performance with Xylose as Sole Sugar



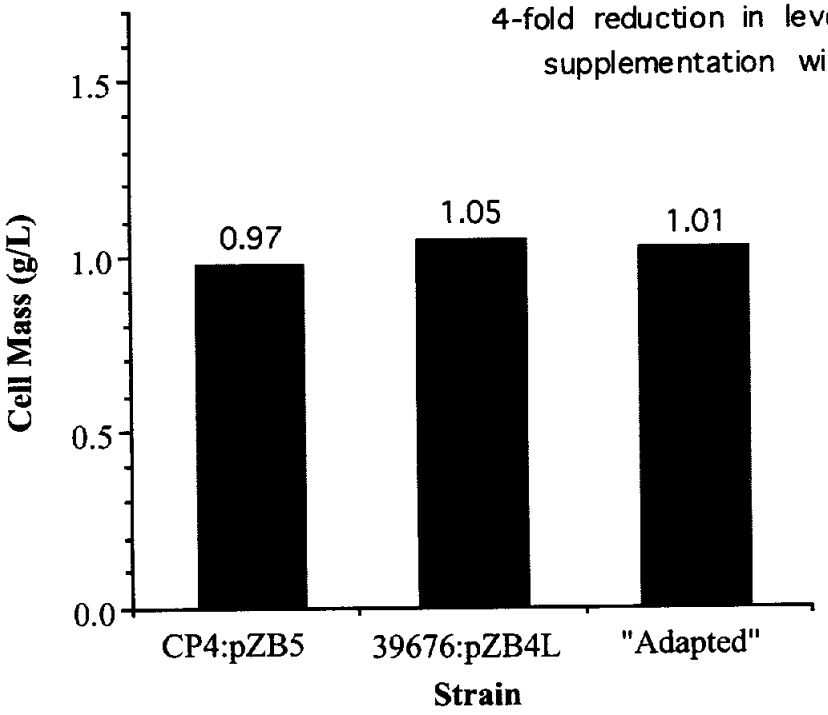
Comparative Growth & Fermentation Performance
Using a Nutrient-rich Medium with 4% Xylose & 0.8% Glucose

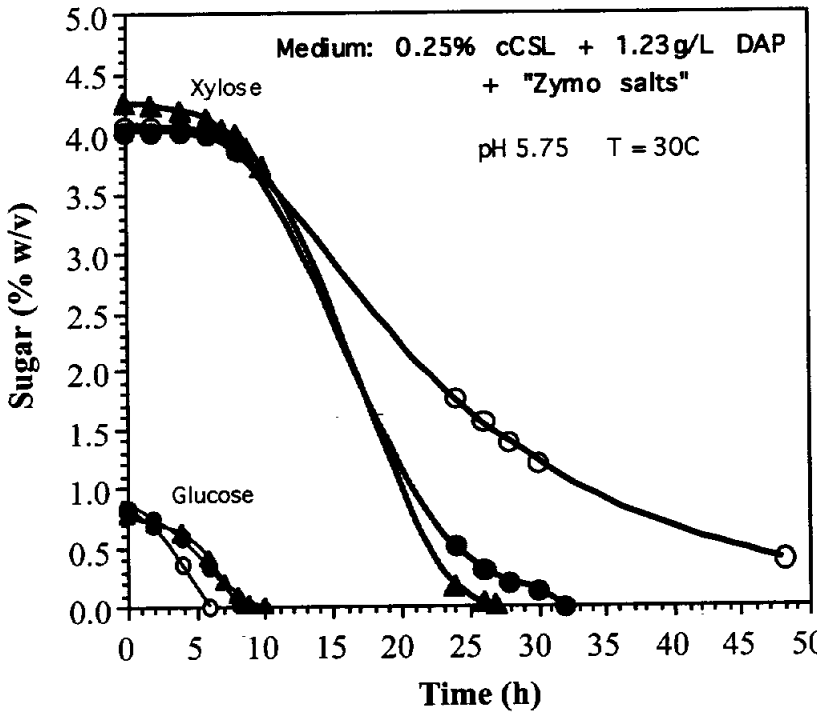




B038a/B112c,d

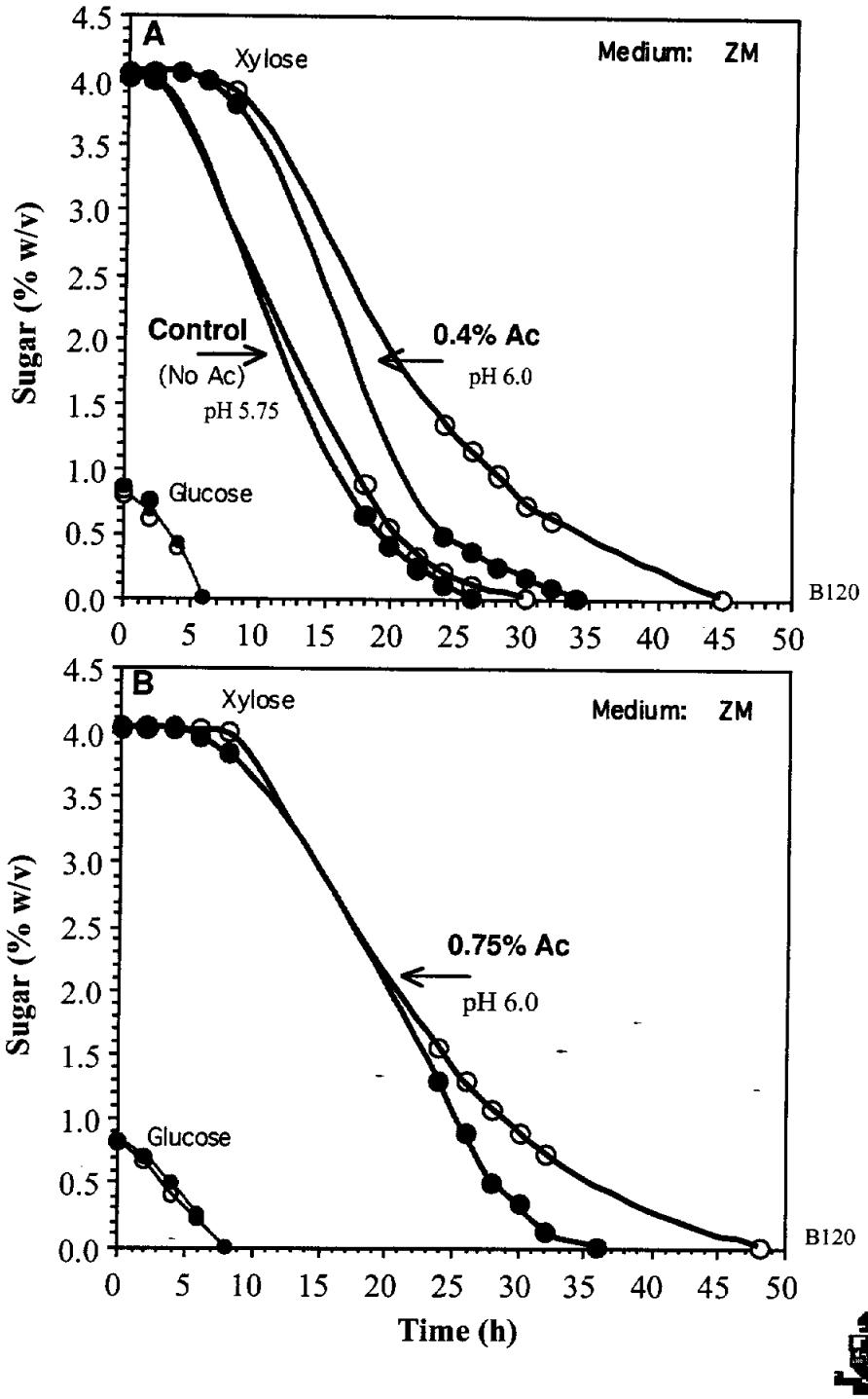
Effect of Replacing CSL with Inorganic Nitrogen



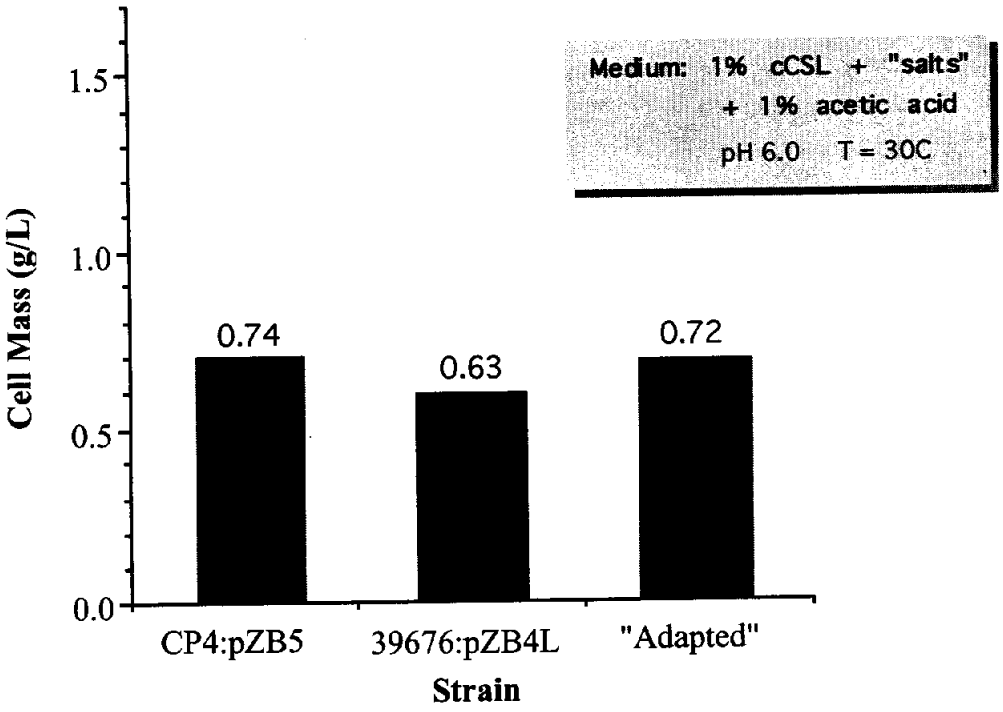


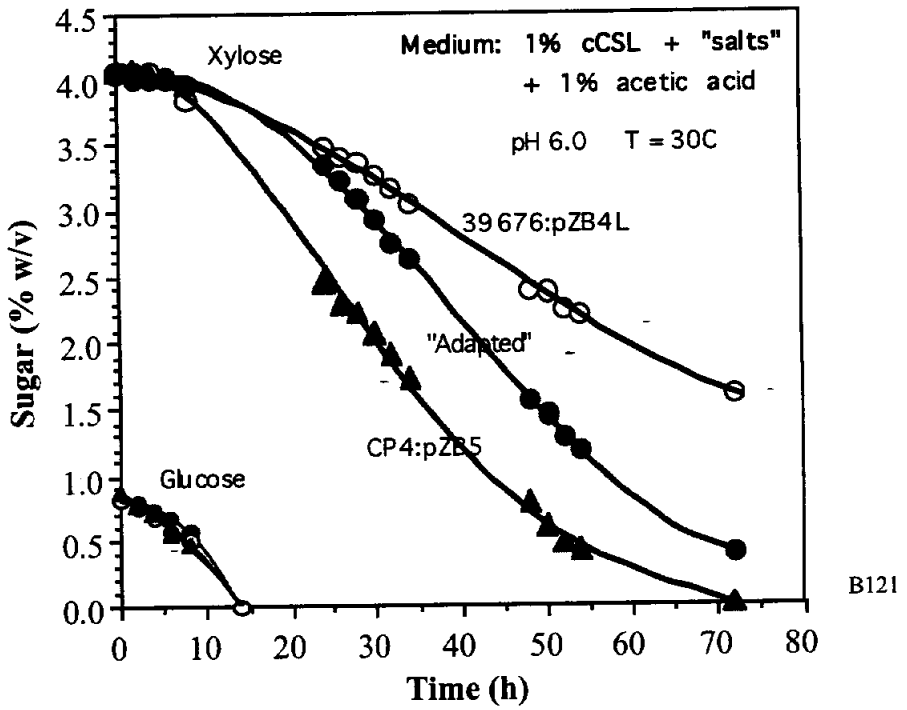
B049d/B118c, d

Comparative Growth & Fermentation Performance in Rich Medium with 0, 0.4% & 0.75% Acetic Acid



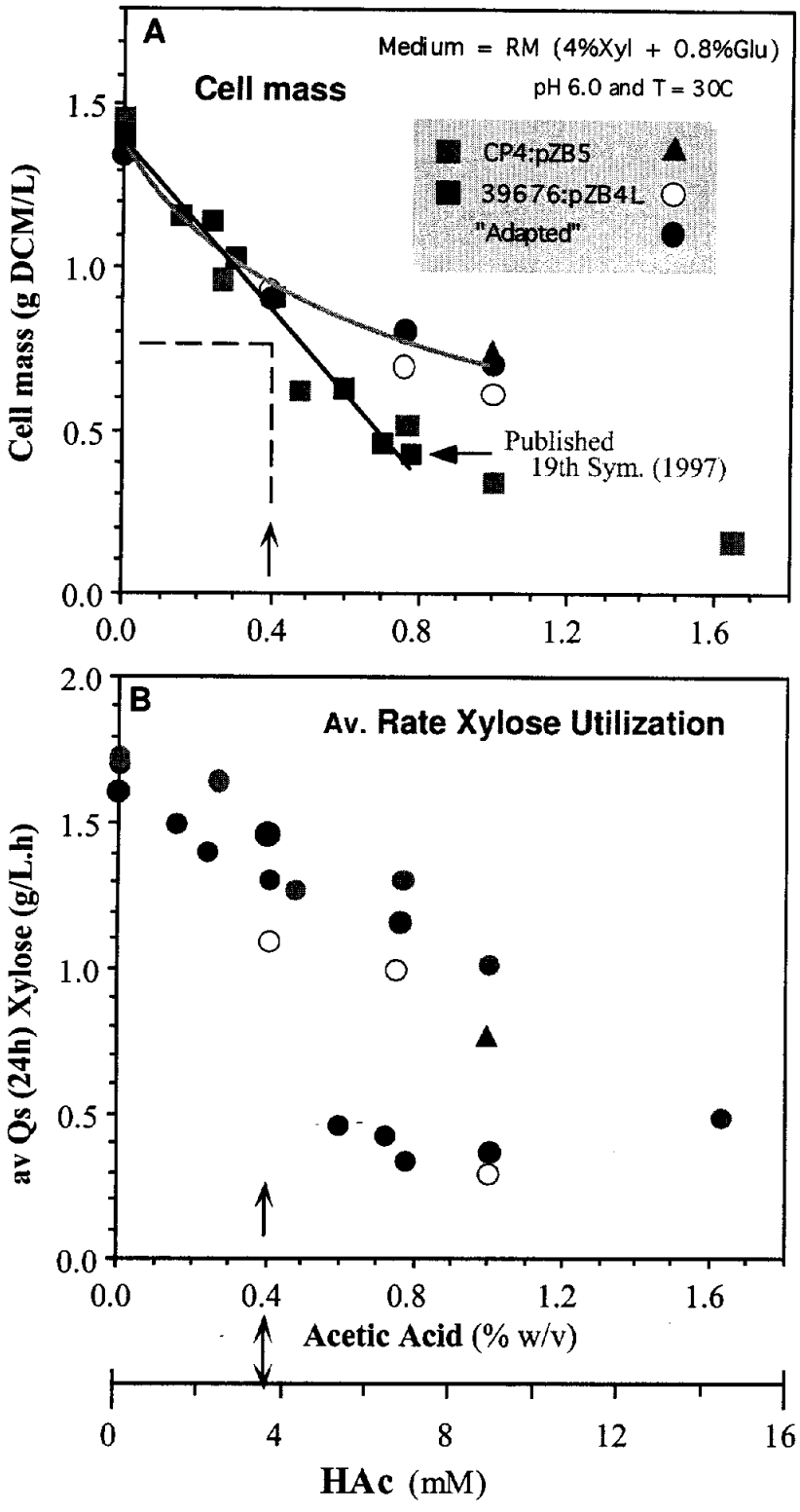
Comparative Growth & Fermentation Performance in cCSL Medium with 1% Acetic Acid



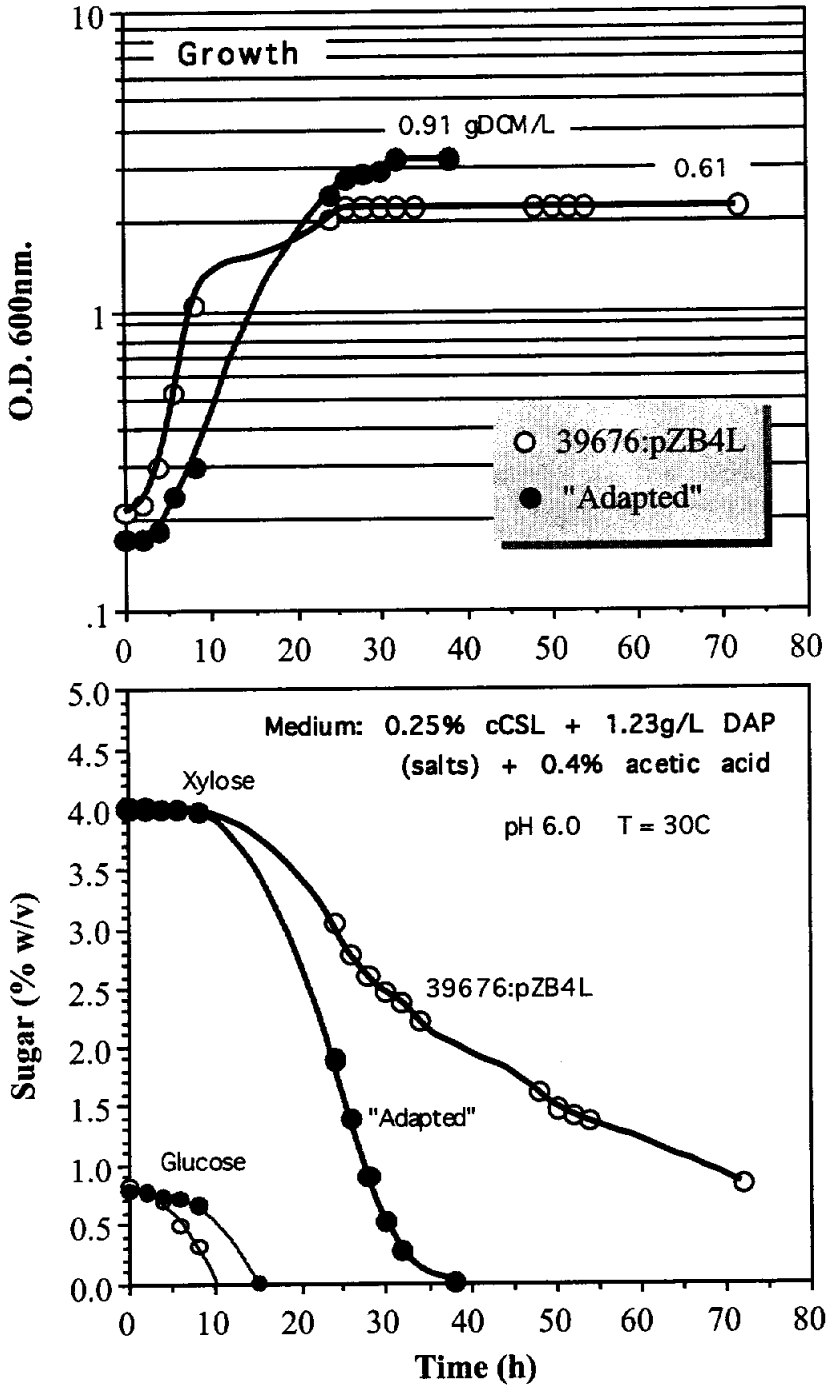


B121

Acetic Acid Sensitivity of rZm CP4:pZB5, 39676:pZB4L and "adapted" variant 39676:pZB4L(A)

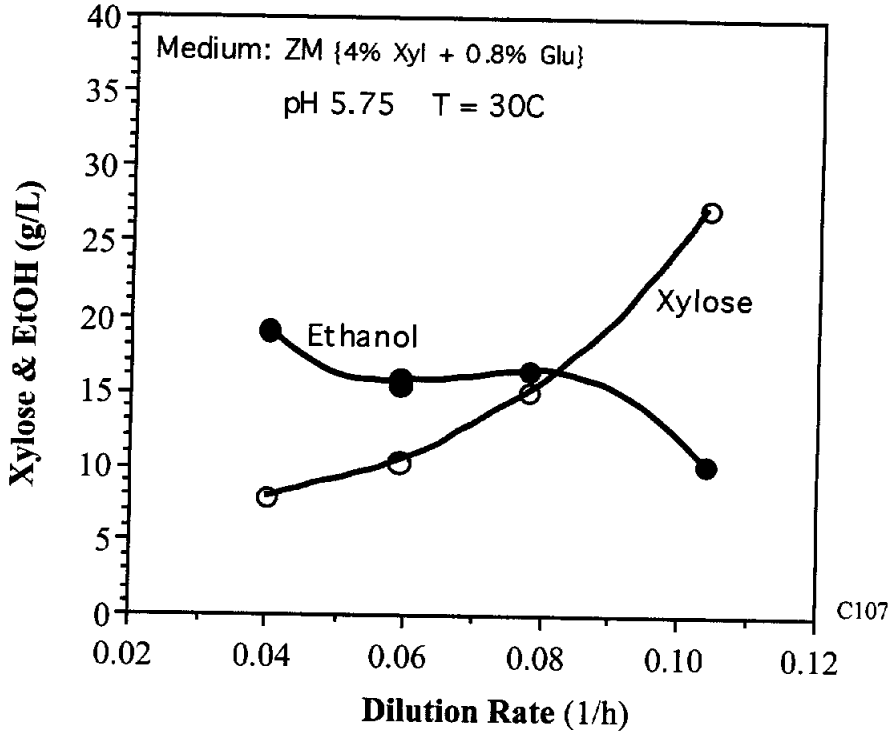


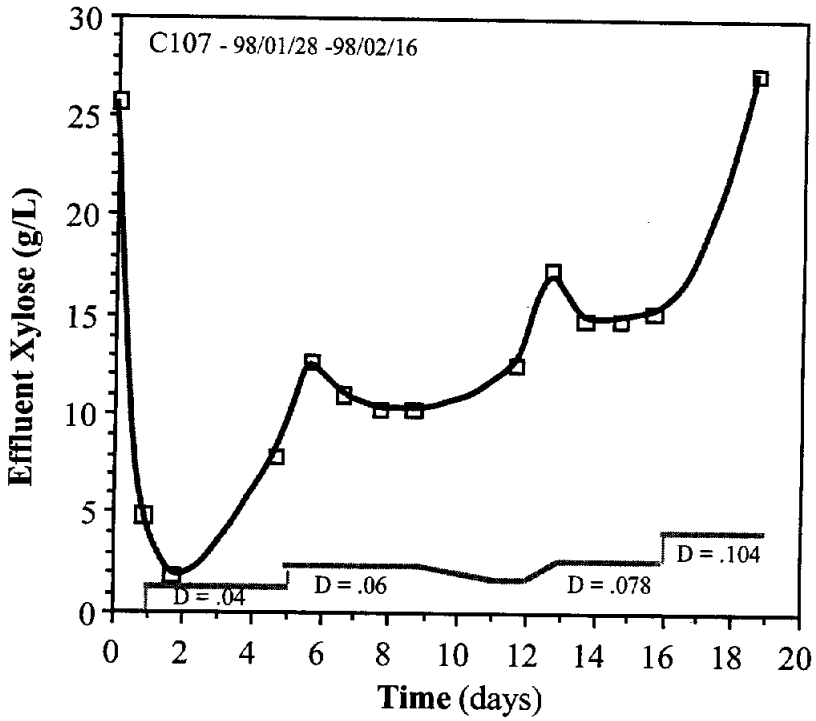
Comparative Growth & Fermentation Performance with reduced cCSL and 0.4% Acetic Acid



B119d
B121a

Continuous culture of "adapted"396767:pZB4L





CONCLUSIONS

1. Using a nutrient-rich medium with glucose as sole sugar, both the growth yield and rate of glucose utilization were slower with the "adapted" variant than for either of the other recombinants

2. With xylose as sole sugar, growth and fermentation were slower with the "adapted" variant compared to the other recombinants - the best being CP4:pZB5

3. Performance by all three rZm strains tested was very similar in the synthetic prehydrolyzate medium containing 40g/L xylose and 8g/L glucose (no Ac) - the batch fermentation being complete in about 24h with a conversion efficiency of 95%

..... continued

CONCLUSIONS ..... continued

4. The cost of CSL is a driver for seeking either a reduction in the amount used or an alternative cost-effective nutrient source. Both the "adapted" and CP4 recombinants perform better than the rec 39676 with 0.25% (v/v) cCSL + ca 1% DAP (even with 0.4% acetic acid)

5. At the 1% (w/v) acetic acid level (pH 6), the rate of xylose utilization is considerably faster with rec CP4 than 39676. The "adapted" strain has a rate which is intermediate between the other strains.

6. Cell mass production in higher levels of acetic acid are enhanced when higher "pitching" is employed (ie. use of larger inoculum)

7. Continuous fermentations with the "adapted" strain are in the early stages and have not yet shown any differences with the non-adapted parent culture rZm 39676:pZB4L

March 2, 1998

Note: The following "CONCLUSIONS" were presented at the NREL Seminar last year (which took place on March 13, 1997)

CONCLUSIONS

1. *Zymomonas* recombinants can utilize xylose as sole fermentable sugar and they are characterized by a very high sugar-to-ethanol conversion efficiency (96-98%).
2. Glucose supplementation of prehydrolyzate is an effective means of increasing cell mass, but the economic impact was not evaluated. The 'physiological state' (history) of the seed culture can affect energetic uncoupling resulting in decreased final cell mass concentration.
3. The composition of CSL is variable. At a volumetric supplementation rate of 1% (v/v), the cost of using cCSL is not negligible and there is a need to identify potential alternative cost-effective nutritional supplements
4. At pH 6.0, there is a 50% reduction in cell mass concentration at an acetic concentration of about 3-4 g/L. Concentrations of HAc >4mM are to be avoided
5. Acetic acid concentration is feedstock specific. Elevating pH reduces acetic acid sensitivity, but promotes a decrease in ethanol yield (especially at low growth rates in rich media)
6. rZm CP4:pZB5 may be more acetic acid resistant than 39676:pZB4L
7. rZm is compatible with long-term continuous culture at low dilution rates and "adaptation" may play a role in capacity for 'near complete' xylose utilization

March 13, 1997

APPENDIX

Supplementary Materials

Figure 1 - 19th Sym. (1997) with new data added

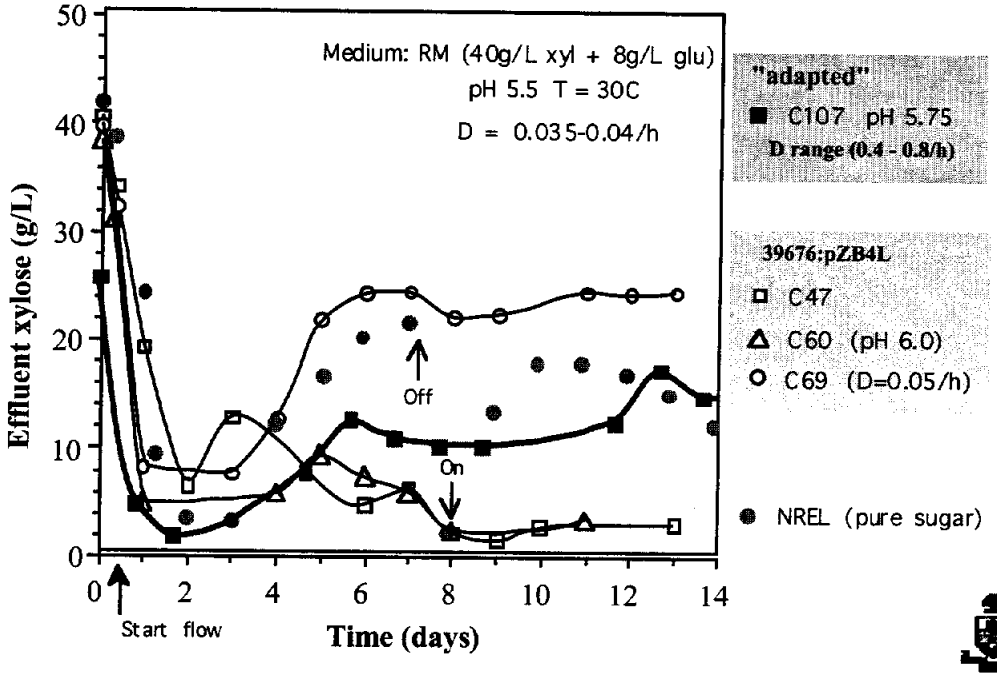
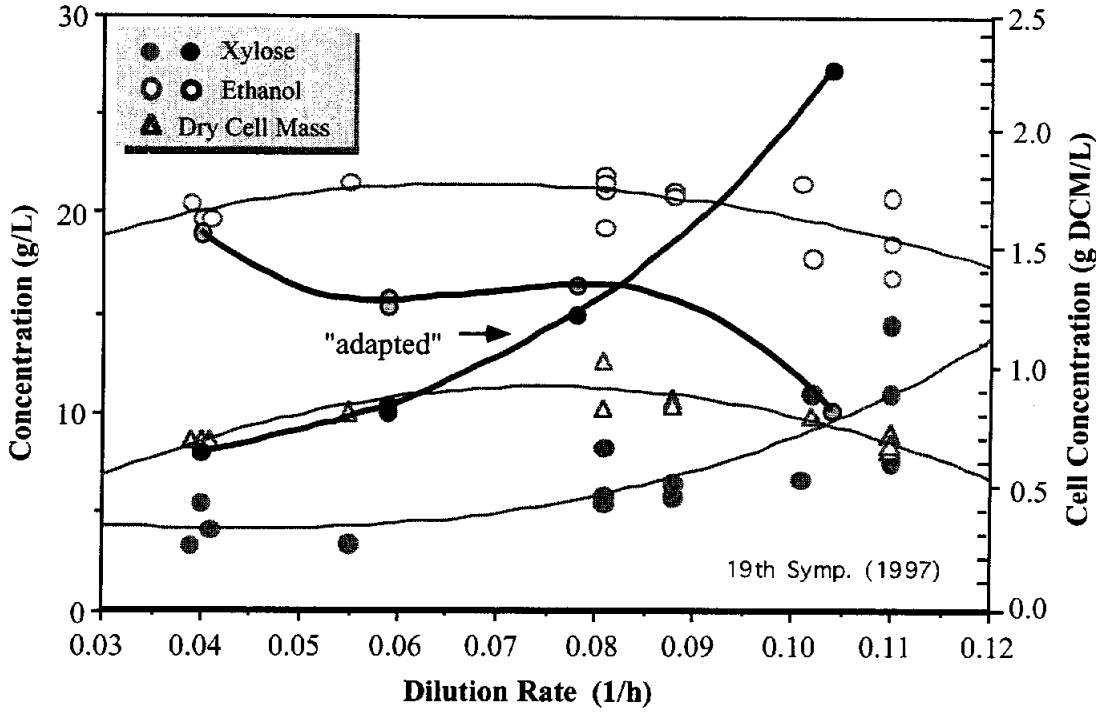
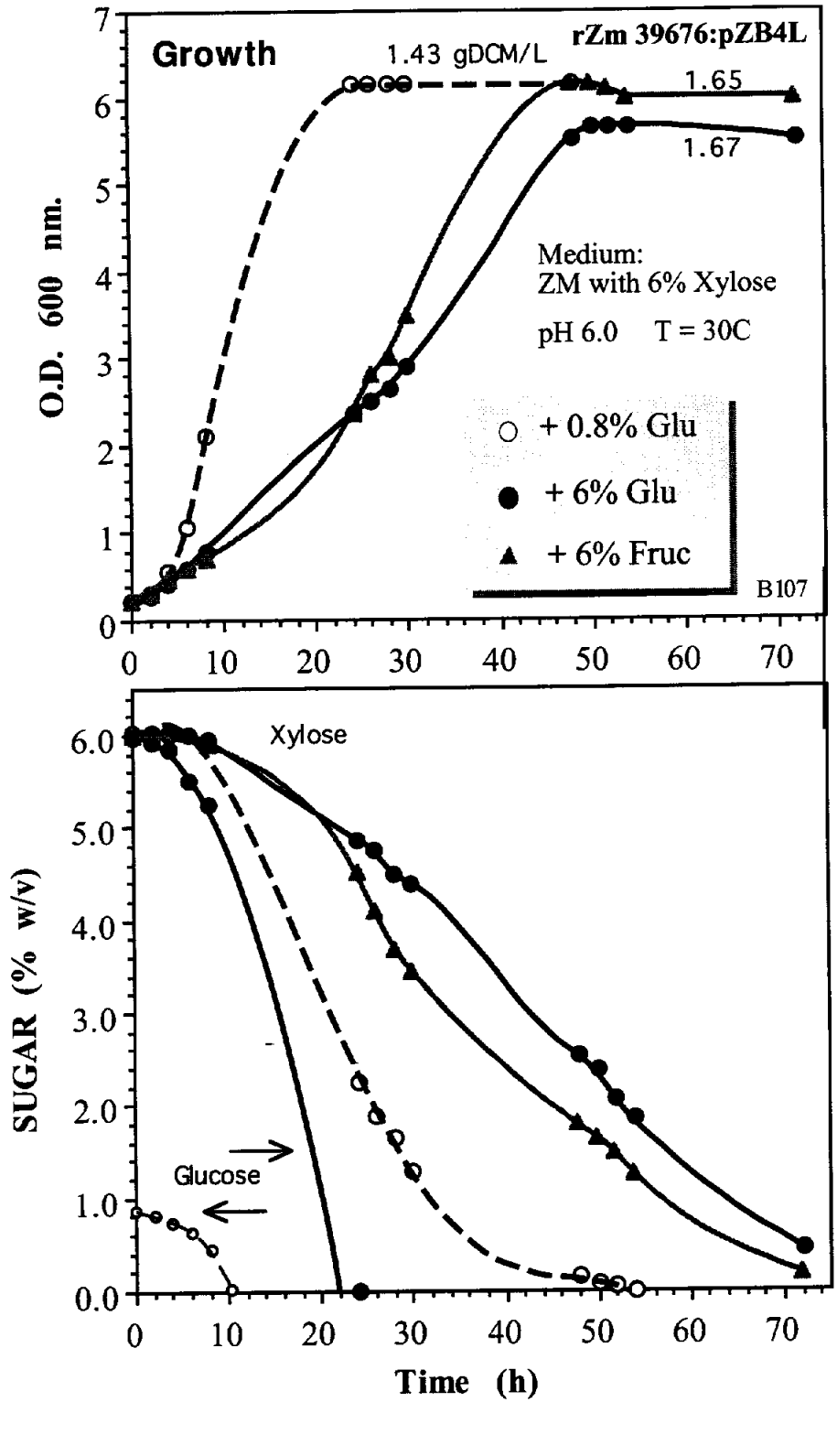


Figure 2: Pure sugar continuous studies

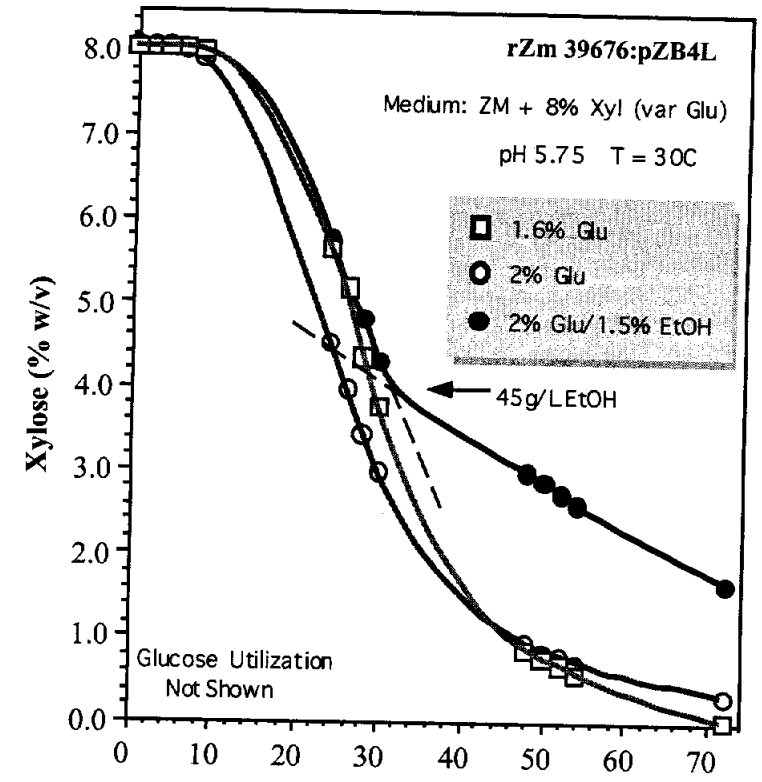


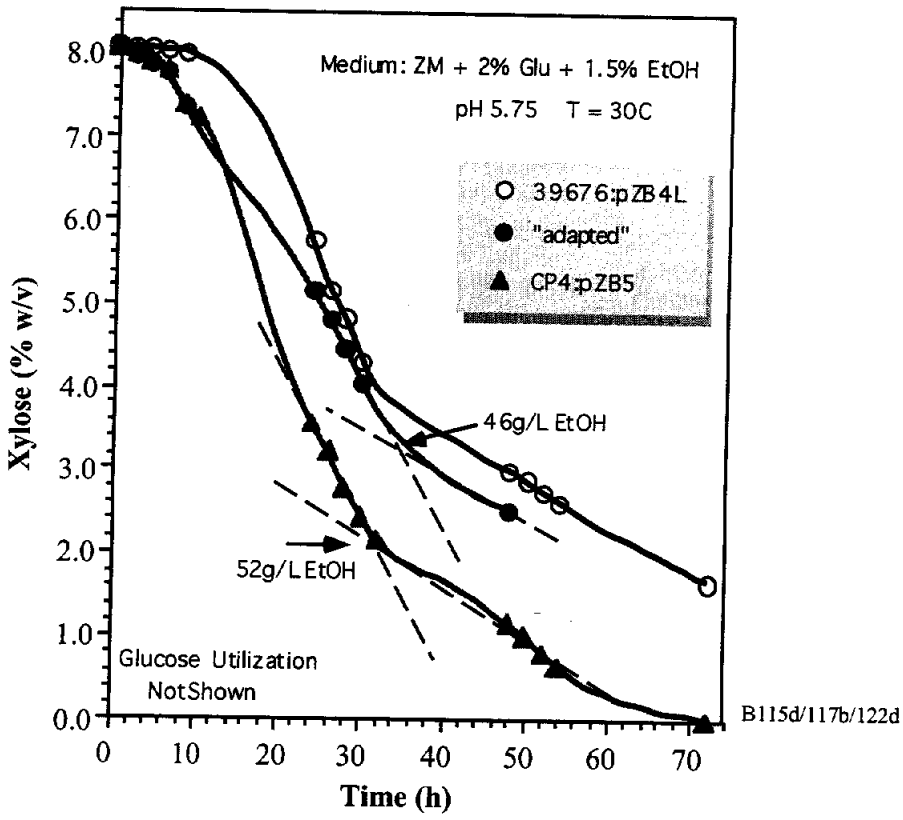
Growth & Fermentation of Rich Medium with 6% (w/v) Xylose Using rZm 39676:pZB4L



Mar 2/98

Effect of Ethanol on Xylose Utilization by Recombinant *Zymomonas*





APPENDIX K

The effect of glucose on high-level xylose fermentations by recombinant *Zymomonas* in batch and fed-batch fermentations

Hugh G. Lawford and Joyce D. Rousseau

Humana Press Inc.

Appl. Biochem. Biotechnol., Vol. 77-79, 1999 (*in press*)

20th Symposium on Biotechnology for Fuels & Chemicals
Gatlinburg, TN, U.S.A.
May 3 - 7, 1998

The Effect of Glucose on High-Level Xylose Fermentations by Recombinant *Zymomonas* in Batch and Fed-Batch Fermentations

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REVISED MANUSCRIPT

for publication in

Applied Biochemistry and Biotechnology

* Author to whom all correspondence should be addressed

ABSTRACT

Xylose-fermenting recombinant *Zymomonas mobilis* has been proposed as a candidate biocatalyst for the production of fuel ethanol from cellulosic biomass and wastes. This study documents the effect of glucose on xylose utilization by recombinant *Z. mobilis* CP4:pZB5 using a nutrient-rich synthetic (pure sugar) hardwood dilute-acid prehydrolyzate medium containing 0.8% (w/v) glucose and 4% (w/v) xylose that was enriched with respect to xylose concentration within the range 6% to 10% (w/v) xylose. Supplementation with glucose to a final concentration of 2% (w/v) resulted in faster xylose utilization of both 6% and 8% xylose; however, higher levels of glucose supplementation (>2%) did not result in a decrease in the time required for fermentation of either 6% or 8% xylose. An improvement in the rate of 8% xylose utilization was also achieved through continuous glucose feeding in which the total glucose concentration was about 1.3% (w/v). This fed-batch experiment was designed to mimic the continuous supply of glucose provided by the cellulose saccharifying enzymes in a simultaneous saccharifying and cofermentation process. The upper limit ethanol concentration at which xylose utilization by recombinant *Z. mobilis* CP4:pZB5 is completely inhibited is about 5.5% (w/v) at pH 5 and >6% at pH 5.75. At pH 5.75, this level of ethanol was achieved with the following media of pure sugar mixtures (each containing the same sugar loading of 12% (w/v): (i) 6% xylose + 6% glucose; (ii) 8% xylose + 4% glucose, and (iii) 4% xylose + 8% glucose. At the level of inoculum used in this study, complete fermentation of the 12% sugar mixtures required 2-3 days (equivalent to a volumetric ethanol productivity of 0.83 - 1.25g ethanol/L.h). The sugar-to-ethanol conversion efficiency was 94-96% of theoretical maximum.

KEY WORDS - recombinant *Zymomonas*, xylose, ethanol tolerance, cofermentation, prehydrolyzate, glucose feeding

Running Title: ***Rec Zymomonas: high xylose***

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INTRODUCTION

Recombinant *Zymomonas mobilis* (rec Zm) carrying genes for xylose metabolism from *E. coli* (1,2) is one of several biocatalysts for the production of ethanol from biomass that is currently under investigation by the National Renewable Energy Laboratory (NREL, Golden, CO) (3-6). In the different simultaneous saccharification and fermentation process designs for the production cellulosic fuel ethanol currently being assessed by NREL, a prerequisite of the biocatalyst is the ability to coferment, in high yield, the major hemicellulosic and cellulosic sugars, namely xylose and glucose, respectively (7,8).We have already demonstrated that recombinant *Zymomonas* has excellent cofermentation characteristics in laboratory synthetic media and in hardwood prehydrolyzate in both batch and continuous fermentations (9,10). In previous work with different Zm recombinants, the majority of pH-stat batch fermentations were conducted with media that contained 5:1 ratio of xylose to glucose, with a maximum xylose concentration of 4% (w/v) (10,11). We demonstrated that the presence of 0.8% glucose in the medium significantly reduced the time required for complete xylose utilization (9). These concentrations of xylose (4% w/v) and glucose (0.8% w/v) were selected to mimic the composition of NREL's dilute-acid hardwood hemicellulose hydrolyzate (12,13). In *Zymomonas*, glucose and xylose compete for the same membrane transporter (14). Since the affinity of the transport system is much higher for glucose than xylose (14), it is reasonable to expect that batch productivity would be affected by the ratio of these two sugars in the medium. Apart from an investigation into the efficacy of glucose feeding on reducing the inhibitory effect of acetic acid on xylose utilization (11), no attempt had been made previously to optimize the level of glucose supplementation for the purpose of effecting maximal rate of xylose utilization. The objective of this study was to examine the effect of glucose on xylose utilization (ie. the time required for complete xylose consumption) using a xylose-enriched synthetic (pure sugar) hardwood prehydrolyzate medium in which the xylose concentration was in the range 6% to 10% (w/v). Furthermore, it was anticipated that these experiments at elevated sugar levels would also afford an opportunity to assess the effect of ethanol on xylose fermentation by recombinant *Zymomonas*.

MATERIALS AND METHODS

Organism

The xylose-utilizing recombinant *Z. mobilis* strain CP4 carrying the plasmid pZB5 (designated as Zm CP4:pZB5) (1,2) was received from M. Zhang (NREL, Golden, CO, USA). Stock cultures were stored in glycerol at -70°C and pre-cultures were prepared as described previously (9).

Preparation of inoculum Overnight flask pre-cultures were harvested by centrifugation (16,300 x g for 10min) and the cell pellet resuspended in RM medium without sugar (15) to yield a concentrated cell suspension that was used to inoculate the batch fermentors. The initial optical density (OD, 1cm light path at 600nm) was in the range 0.2-0.25 corresponding to 60-75 mg dry cell mass (DCM) per liter.

Fermentation medium and equipment

The fermentation medium (designated as "ZM") was prepared with glass distilled water and contained the following ingredients: 5g/L Difco Yeast Extract (YE) (Difco Laboratories, Detroit, MI); 3.48g/L KH_2PO_4; 0.8g/L NH_4Cl; 0.5g/L $MgSO_4$; 0.01g/L $FeSO_4.7H_2O$; 0.21g/L citric acid; 20mg/L tetracycline. The amount of glucose and xylose added to the medium was variable. The medium and stock sugar solutions were autoclaved separately. Batch and fed-batch fermentations were conducted with about 1500ml medium in 2L bioreactors (model F2000 MultiGen, New Brunswick Scientific, Edison, NJ) fitted with agitation (100 RPM), pH, and temperature control (30°C). The pH was monitored using a sterilizable combination pH electrode (Ingold). The standard pH control set-point was 5.75 and the pH was kept constant by the automatic titration with 4N KOH. In fed-batch fermentations, a peristaltic pump was used to deliver 4.85% (w/v) glucose at a constant rate of 2mL/h through the central agitator shaft of the bioreactor; the flow rate was determined with the aid of an in-line pipette. The glucose feed was initiated after 8h elapsed fermentation time when most of the initial 0.8% glucose had been consumed.

Analytical procedures, growth and fermentation parameters

Growth was measured turbidometrically at 600nm (1 cm light path) (Unicam spectrophotometer, model SP1800). In all cases the blank cuvette contained distilled water. Dry cell mass (DCM) was determined by microfiltration of an aliquot of culture followed by washing and drying of the filter to

constant weight under an infrared heat lamp. Fermentation media and cell-free spent media were compositionally analyzed by HPLC as described previously (9). The ethanol yield ($Y_{p/s}$) was calculated as the mass of ethanol produced per mass of sugar consumed. The volumetric ethanol productivity was determined by dividing the final ethanol concentration by the total batch fermentation time.

RESULTS and DISCUSSION

Growth and fermentation performance of recombinant Zm in xylose-enriched synthetic hardwood prehydrolyzate medium

In previous work we used a synthetic biomass (hardwood) prehydrolyzate medium (BPH) to assess the growth and cofermentation performance characteristics of recombinant *Z. mobilis* CP4:pZB5 (9) and 39676:pZB4L (11). The nutrient-rich synthetic BPH media were formulated to model the sugar concentration in the NREL hardwood (yellow poplar) dilute-acid prehydrolyzate (12,13) and contained 4% (w/v) xylose and 0.8% (w/v) glucose (9,11). In this work, the formulation of the synthetic BPH was modified slightly by reducing the level of yeast extract 50% and by the addition of both ammonium and magnesium salts (see *Materials & Methods*). Furthermore, in this work the pH was controlled slightly lower at 5.75 compared to 6.0 previously. However, these modifications did not significantly alter the growth and cofermentation performance of recombinant CP4:pZB5 (Fig. 1; open circle symbol) relative to what had been observed previously with 0.8% glucose and 4% xylose (9). The almost superimposable optical density trajectories in Figure 1A show that increasing the xylose loading of the BPH medium to give final xylose concentrations of 6% (w/v) and 8% (w/v) did not appreciably affect either the growth rate or yield. The final cell mass concentrations for the standard 4% xylose BPH, 6% and 8% media were 1.39, 1.46 and 1.48 g DCM/L, respectively (Table 1). Growth was affected when the media contained 10% xylose (Fig 1A); in this case the final cell concentration was reduced 1.18 g DCM/L (Table 1).

Using the standard 4% xylose BPH medium, what is typically observed is that the rate of xylose utilization slows exponentially when the residual xylose concentration falls below about 0.5% (w/v) (Fig 1B) and this is most probably due to the relative low affinity of the membrane transporter for xylose compared to glucose (14). This "tailing off" phenomenon with respect to xylose utilization is a characteristic of recombinant Zm and is independent of any inhibitory effect of ethanol (1,8,9,11).

Utilization of the 0.8% glucose is progressively protracted at increasing levels of xylose supplementation (Fig. 1B). This pattern is consistent with both sugars entering the cell by a common membrane transporter (14). The medium with 6% xylose was completely fermented in 48h (Fig 1B). Previously, we had observed with another recombinant strain that when 2% xylose was added to the medium following the almost complete fermentation of the synthetic BPH, the xylose was completely utilized within 48h (11). Neither the 8% or 10% xylose media were finished when the experiment was terminated at 54h (Fig 1B), although in both cases the final ethanol concentration (Fig. 1C, Table 1) was well below what might be considered "inhibitory" (16). The ethanol productivity associated with all these batch fermentation was very similar (Table 1). This may be a consequence of the similarity in cell density for these fermentations (Table 1). The ethanol yield was 0.48g ethanol/g sugars consumed, which represents 94% of the theoretical maximum sugar-to-ethanol conversion efficiency (Table 1). This series of batch fermentations with 0.8% glucose and increasing amounts of xylose showed the potential for improving the productivity through an increase in the rate of xylose utilization, especially with higher levels of xylose.

Fermentation of 6% xylose supplemented with different amounts of glucose

In this series of pH-stat batch fermentations, we explored the effect of increasing levels of glucose supplementation of a medium containing 6% xylose. In the absence of glucose, the recombinant grows relatively slowly (Fig 2A) and the final cell mass concentration is 0.74 g DCM/L (Table 1). Slow growth in the absence of glucose is a recognized characteristic of this recombinant (1), but it was interesting to note that the final cell concentration with 6% xylose was very similar to what was observed previously with either 2.5% (unpublished results) or 4% xylose (9). This phenomenon of growth limitation cannot be explained in terms of nutrient limitation since the medium used was nutrient rich and clearly capable of supporting higher cell mass concentrations than the limit of 0.74 achieved with xylose as the sole sugar and energy source. Hence the explanation of this observation remains problematic.

Supplementation of the 6% xylose medium with glucose resulted in faster growth (Fig 2A) and a final cell mass concentration that was proportional to the amount of glucose added (Table 1). In the absence of glucose supplementation, about 1.5% xylose remained unconsumed when the experiment was terminated at 72h, whereas all the xylose was completely consumed in 48h when 0.8% glucose was added to the 6% xylose medium (Fig. 2C). The rate of xylose utilization is improved by the addition

of 2% glucose to the medium; however, levels of glucose supplementation >2% caused the time required for complete 6% xylose utilization to increase from 48h to >60h (Fig. 2C). In the case of supplementation at the level of 4% and 6% glucose, the utilization of xylose may be initially retarded because of competitive inhibition of xylose uptake by glucose.

In the experiment with 6% xylose and 6% glucose, complete xylose utilization was achieved after 62h (Fig. 2C). The final ethanol concentration was 5.9% (w/v) (Fig. 2D), which represents an ethanol yield of 0.49g/g or a sugar conversion efficiency of 96% of theoretical maximum (Table 1). This observation with respect to maximum ethanol concentration has particular relevance in terms of the recent suggestion made by Rogers *et al.* (16) regarding this recombinant: "...at least one of the enzymes, which has been cloned into *Z. mobilis* to facilitate pentose metabolism, has been fully inhibited by 55g/L ethanol." (p305) In another section of this study we have further explored the effect of ethanol on xylose fermentation.

This series of batch fermentations demonstrate that the time required by the recombinant (at the inoculation level employed) to ferment 6% xylose can be significantly reduced through glucose supplementation and furthermore they suggest that the optimal effect is achieved within the range of 0.8-2% (w/v) glucose.

Fermentation of 8% xylose supplemented with different amounts of glucose

In this series of pH-stat batch fermentations, we explored the effect of increasing levels of glucose supplementation of a medium containing 8% xylose. We were interested to see if the same enhancing effect of glucose on xylose utilization that had been observed with 6% xylose could be achieved at higher levels of xylose.

Supplementation of the 8% xylose medium with 0.8%, 2% and 4% glucose resulted in proportionately higher final cell mass concentrations (Fig. 3A, Table 1). With the addition of 0.8% glucose, 1.2% xylose remained unconsumed after 72h whereas with 2% glucose added to the medium, the 8% xylose was completely fermented to ethanol (yield = 0.48g/g) in 72h (Fig. 3B). Increasing the amount of glucose from 2% to 3% (not shown) or 4% did not shorten the time required for complete xylose utilization (Fig. 3B). With 4% glucose, the final ethanol concentration was 5.7% (w/v) (Fig. 3C, Table 1) and this level of ethanol may have contributed to a retardation of xylose utilization towards the end of the fermentation (Fig. 3B). It was concluded that xylose utilization can

be enhanced by means of glucose supplementation with the level of 2% glucose producing the fastest utilization of the 8% xylose.

In another related experiment, that was part of a separate study (data not plotted), we observed that a mixture of 4% xylose and 8% glucose was completely fermented in 60h with a final ethanol concentration of 5.82% (w/v) and volumetric productivity of 0.97g/L/h (Table 1). With this mixture the final cell density was 2.37 g DCM/L; the 8% glucose was completely fermented in 34h and the 4% xylose was completely utilized in 60h (Table 1). Like the experiment with the 8% xylose and 4% glucose (Fig. 3C), the relatively high level of ethanol generated from the 8% glucose and 4% xylose medium may have protracted the time required to complete the fermentation by inhibitiing xylose utilization in the final stages of the batch fermentation (50-60h) (results not shown).

In the simultaneous cofermentation (SSCF) process design proposed by NREL (5,7) the saccharification of cellulose would provide a continuous supply of glucose to the ethanologenic biocatalyst. In order to model this situation whereby the continuous supply of glucose might not be expected to cause the same level of competitive inhibition of xylose uptake produced by the higher levels of glucose supplementation previously tested, we performed a fed-batch experiment in which glucose feeding was initiated after the 0.8% glucose had been consumed in batch mode. The feed reservoir contained 4.85% (w/v) glucose and the feed rate was constant at 2ml/h over the period from 8h to 72h (Fig 3; open triangle symbol). In the fed-batch experiment, the level of glucose supplementation was equivalent to 1.28% (w/v) (0.8% + 0.48%). Glucose feeding promoted growth beyond the level observed with the standard BPH medium (Fig. 3A); the final cell mass concentration was 1.66 g DCM/L (Table 1). However, perhaps most significant was the observation that the xylose utilization trajectories for the 2% glucose supplemented medium and the fed-batch fermentation were very similar (Fig. 3B). In a previous study with recombinant Zm we demonstrated the benefit of providing a continuous supply of glucose for xylose fermentation in reducing the effect of inhibition by acetic acid (11). The results of the present work with the fed-batch fermentation auger well for the performance of recombinant Zm in the simultaneous saccharification cofermentation process for the production of cellulosic ethanol where the xylose concentration is expected to be in the range 4 to 6%.

Effect of ethanol on xylose utilization

Wild-type *Zymomonas* is known to be as ethanol tolerant as yeast (17-20); however, xylose utilizing recombinant *Zymomonas* appears to be more sensitive to end-production inhibition in terms of xylose fermentation (16). This study at elevated levels of xylose provided an opportunity to assess the effect of ethanol on xylose utilization by recombinant Zm CP4:pZB5. Both the fermentation with 6% xylose and 6% glucose and the fermentation with 8% xylose and 4% glucose produced final ethanol concentrations which exceeded the xylose fermentation limit of 5.5% (w/v) (Table 1) proposed recently by Rogers *et al.* for this recombinant strain (16).

Since it had been demonstrated that the recombinant produced 4.8% (w/v) ethanol from a medium containing 8% xylose and 2% glucose, the addition of 1.5% (w/v) ethanol to a medium containing 8% xylose and 2% glucose should in theory yield a final ethanol concentration of about 6.3% (w/v) (4.8% + 1.5%). Figure 4A shows that addition of 1.5% (w/v) ethanol had only a slight inhibitory effect on growth; the final cell mass concentration fell from 1.79 to 1.73 g DCM/L (Table 1). Likewise, this level of exogenous ethanol did not appreciably affect glucose utilization (Fig. 4B); however, it did inhibit xylose utilization (Fig. 4B). In the absence of added ethanol the 8% xylose was completely fermented in 72h, but in the presence of 1.5% (w/v) exogenous ethanol, about 1.4% xylose remained unconsumed at 72h (Fig. 4B) when the ethanol concentration was 5.6% (w/v) (Fig. 4C, Table 1). The inhibitory effect of ethanol was already apparent at 24h when the ethanol concentration was approximately 4% (w/v) (Fig.4B and 4C). In the absence of added ethanol, the control fermentation with 8% xylose and 2% glucose does not achieve a level of 4% ethanol until about 40h elapsed fermentation time (Fig. 4C).

Cofermentation by recombinant Zm of 6.5% xylose and 6.5% glucose

The results of the experiment with 6% xylose and 6% glucose that are shown in Figure 2 are very similar to the observations reported last year by Rogers *et al.* (16) using this same recombinant and medium; however, whereas our experiments were performed at pH 5.75, it was noted that their experiment was performed with the pH controlled at 5.0 (16). At a sugar-to-ethanol conversion efficiency of 94% (equivalent to an ethanol yield of 0.48g/g), the expected yield of ethanol from a mixture of 6.5% xylose and 6.5% glucose is 6.24% (w/v) ethanol. This level of ethanol is higher than had been observed in our previous experiments. It was not known to what extent the pH might affect

either the rate of xylose utilization or the final ethanol concentration. Figure 5A shows that the final cell mass concentrations at pH 5.0 and 5.75 are similar. Utilization of glucose is slightly faster at the higher pH (Fig. 5B). The utilization of xylose appears to be affected by the difference in pH only towards the end of the fermentation as the ethanol concentration rises above 5% (w/v) (Fig. 5B). We chose to control the pH at the higher set-point in our experiments with a view to reducing the inhibitory effect of acetic acid that is present in hardwood dilute-acid prehydrolyzate (9,21). However, at more tolerable levels of acetate brought about by some treatment that removes acetic acid from the prehydrolyzate or alternatively through the use of an acetate-tolerant mutant (22), operation at the lower pH of 5 would be beneficial to the proposed SSCF process to better accommodate the pH optimum of the cellulose saccharifying enzymes (7).

In the context of the effect of ethanol on xylose utilization by the recombinant, it is most interesting to note that the fermentation appeared to stall with a residual of about 1% xylose at pH 5.0 when the ethanol concentration reached 5.5% (w/v) after 32h, whereas at pH 5.75, all the xylose was consumed and the ethanol concentration was 6.2% (w/v) after 48h (Fig. 5B). These experiments suggest that, for this rec Zm strain, the upper limit concentration of ethanol, at which xylose utilization is completely inhibited, is pH-dependent. The experiments leading to the previously proposed limit ethanol concentration of 5.5% were performed at pH 5.0 (16).

At this meeting Joachimsthal *et al.* (23) reported that another NREL-generated recombinant Zm strain, namely rec Zm 31821:pZB5 (also known as "ZM4:pZB5") can completely ferment a mixture of 6.5% xylose and 6.5% glucose in 48h (at 30°C and pH 5.0) producing "in excess of 60g/L"; "the yield based on sugars available was in excess of 90% of theoretical" (Abstract #10). *Z. mobilis* ZM4 (ATCC 31821) is the subject of several patents (24,25) and is claimed to be superior to other wild-type strains with respect to several key process techno-ethanologenic traits (17). The volumetric ethanol productivity of 1.25g/L/h exhibited by ZM4:pZB5 is similar to that observed in our present study under similar experimental conditions and sugar loading (Table 1). Although it may well be that, because of its pH optimum and ethanol tolerance, *Z. mobilis* ZM4 proves to be a more robust host for pentose metabolic engineering, the present study points to the importance of making performance comparisons under identical conditions since at pH 5.75 rec CP4:pZB5 appears to perform as well as rec ZM4:pZB5 at pH 5 (23).

Finally, it is recognized that recombinant *Zymomonas* is not the only candidate biocatalyst for the production of biomass-derived fuel ethanol (for review see ref. 26). Other promising metabolically engineered ethanologenic biocatalysts include *E. coli* strains KO11 (27) and SL40 (28), *K. oxytoca* M5A1 (29), and the xylose-fermenting yeast recombinant *S. cerevisiae* 1400(pLNH33) (30). In a review of recent data concerning the xylose fermentation and cofermentation performance characteristics of these different recombinants, Rogers *et al.* (16) point out that: "all have comparable ethanol tolerances at this stage of development (viz., 55-60g/L) as well as similar yields and productivities." (p306) A proper comparison of productivities is made difficult because of the influence of inoculum size; however, techno-economic sensitivity analyses have shown ethanol yield to be the prime factor in the cost of cellulosic ethanol production (31,32) From this perspective, it is perhaps noteworthy that the most recent published information concerning cofermentation of xylose and glucose by recombinant *S. cerevisae* 1400(pLNH33) indicates that the conversion efficiency is only 80% of theoretical (33) Therefore, at least with respect to yield, it appears that recombinant *Zymomonas* is superior. Nevertheless, we agree with Rogers *et al.* (16) in their statement that "other factors (that are) likely to influence the final selection of the optimal strain include strain stability, resistance to inhibitors, susceptibility to contamination and safety/regulatory issues related to large-scale fermentations with recombinant microorganisms." (p306).

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Figure Captions

Fig. 1 Fermentation of synthetic hardwood prehydrolyzate fortified with different amounts of xylose (**A**) Growth, (**B**) glucose and xylose utilization, and (**C**) ethanol production. The ZM medium (see *Methods*) contained 0.8% (w/v) glucose and was supplemented with 4, 6, 8 or 10% (w/v) xylose. The temperature was kept constant at 30°C. The pH-control set-point was 5.75. The maximum dry cell mass concentrations and values for both ethanol yield and productivity are given in Table 1.

Fig. 2 Fermentation of 6% xylose medium supplemented with different amounts of glucose. (**A**) Growth, (**B**) glucose utilization, (**C**) xylose utilization, and (**D**) ethanol production. The ZM medium contained 6% (w/v) xylose and was supplemented with 0, 0.8, 2, 4 or 6% (w/v) glucose. The temperature was 30°C and the pH was 5.75. The maximum dry cell mass concentrations and values for both ethanol yield and productivity are given in Table 1.

Fig. 3 Fermentation of 8% xylose medium supplemented with different amounts of glucose. (**A**) Growth, (**B**) glucose and xylose utilization, and (**C**) ethanol production. The ZM medium contained 8% (w/v) xylose and was supplemented with 0.8, 2 or 4% (w/v) glucose. In the fed-batch experiment (open triangles), the glucose feed (4.85%) was started after 8h; the feed rate was 2ml/h and continued until the fermentation was terminated at 72h. The temperature was 30°C and the pH was 5.75. The maximum dry cell mass concentrations and values for both ethanol yield and productivity are given in Table 1.

Fig. 4 Effect of 1.5% (w/v) exogenous ethanol on the fermentation of 8% xylose and 2% glucose by recombinant Zm CP4:pZB5 (**A**) Growth, (**B**) glucose and xylose utilization, and (**C**) ethanol concentration. The ZM medium contained 8% (w/v) xylose and was supplemented with 0.8 or 2% (w/v) glucose. In the experiment with added ethanol (open circles), the medium contained 8% xylose, 2% glucose and 1.5% (w/v) ethanol. The temperature was 30°C and the pH was 5.75. The maximum dry cell mass concentrations and values for both ethanol yield and productivity are given in Table 1.

Fig. 5 Cofermentation of 6.5% xylose and 6.5% glucose at pH 5.0 and 5.75 (**A**) Growth, (**B**) glucose and xylose utilization, and (**C**) ethanol production. The ZM medium contained equal concentrations (6.5% w/v) of xylose and glucose. The temperature was 30°C and the pH was controlled at either 5.0 (open circles) or 5.75 (filled circles). The maximum dry cell mass concentrations and values for both ethanol yield and productivity are given in Table 1.

5 FIGURES and 1 TABLE

Table 1 Summary of growth and fermentation parameters

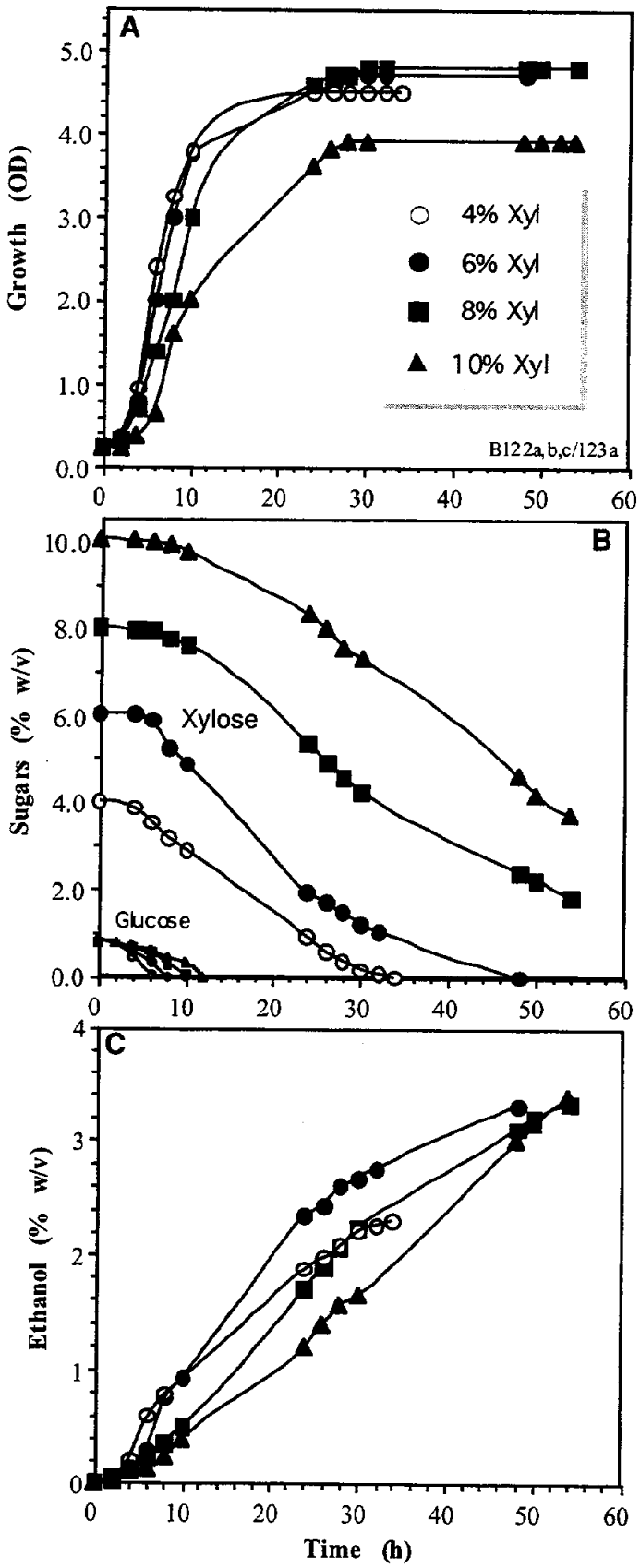
Medium (sugar conc'n)		Maximum	Maximum	Ethanol	Ethanol
Xylose	Glucose	Cell Mass	Ethanol	Yield	Productivity
% (w/v)	% (w/v)	(g DCM/L)	(g/L)	(g/g)	(g EtOH/L/h)
Expts. for Figure 1					
4	0.8	1.39	23.0	0.48	0.68
6	0.8	1.46	33.0	0.48	0.69
8	0.8	1.48	33.6	0.48	(0.62)
10	0.8	1.18	33.9	0.47	(0.63)
Expts for Figure 2					
6	0	0.74	23.0	0.48	(0.32)
6	0.8	1.46	33.0	0.48	0.69
6	2	1.69	39.7	0.49	0.83
6	4	1.85	49.5	0.49	0.69
6	6	2.03	58.6	0.49	0.95
Expts. for Figure 3					
8	0.8	1.48	36.6	0.48	(0.51)
8	2	1.79	47.7	0.48	0.66
8	4	2.03	57.2	0.48	0.79
4	8	2.37	58.2	0.49	0.97
Fed-batch (0.48 % w/v Glc added over 64h)					
8	0.8	1.66	44.7	0.48	0.62
Expts. for Figure 4					
8	2	1.79	47.7	0.48	0.66
8*	2*	1.73	56.2	0.48	(0.57)
Expts. for Figure 5					
6.6	6.7	2.46	61.9	0.46	1.29
6.5•	6.5•	2.44	58.0	0.47	1.18

• pH 5.0

* 1.5% (w/v) ethanol added

Brackets around values for Ethanol Productivity indicate that xylose utilization was incomplete when batch fermentation was terminated

FIGURE 1


A
Growth (OD)
5.0
4.0
3.0
2.0
1.0
0.0
0
10
20
30
40
50
60
4% Xyl
6% Xyl
8% Xyl
10% Xyl
B122a,b,c/123a
B
Sugars (% w/v)
10.0
8.0
6.0
4.0
2.0
0.0
Xylose
Glucose
C
Ethanol (% w/v)
4
3
2
1
0
Time (h)

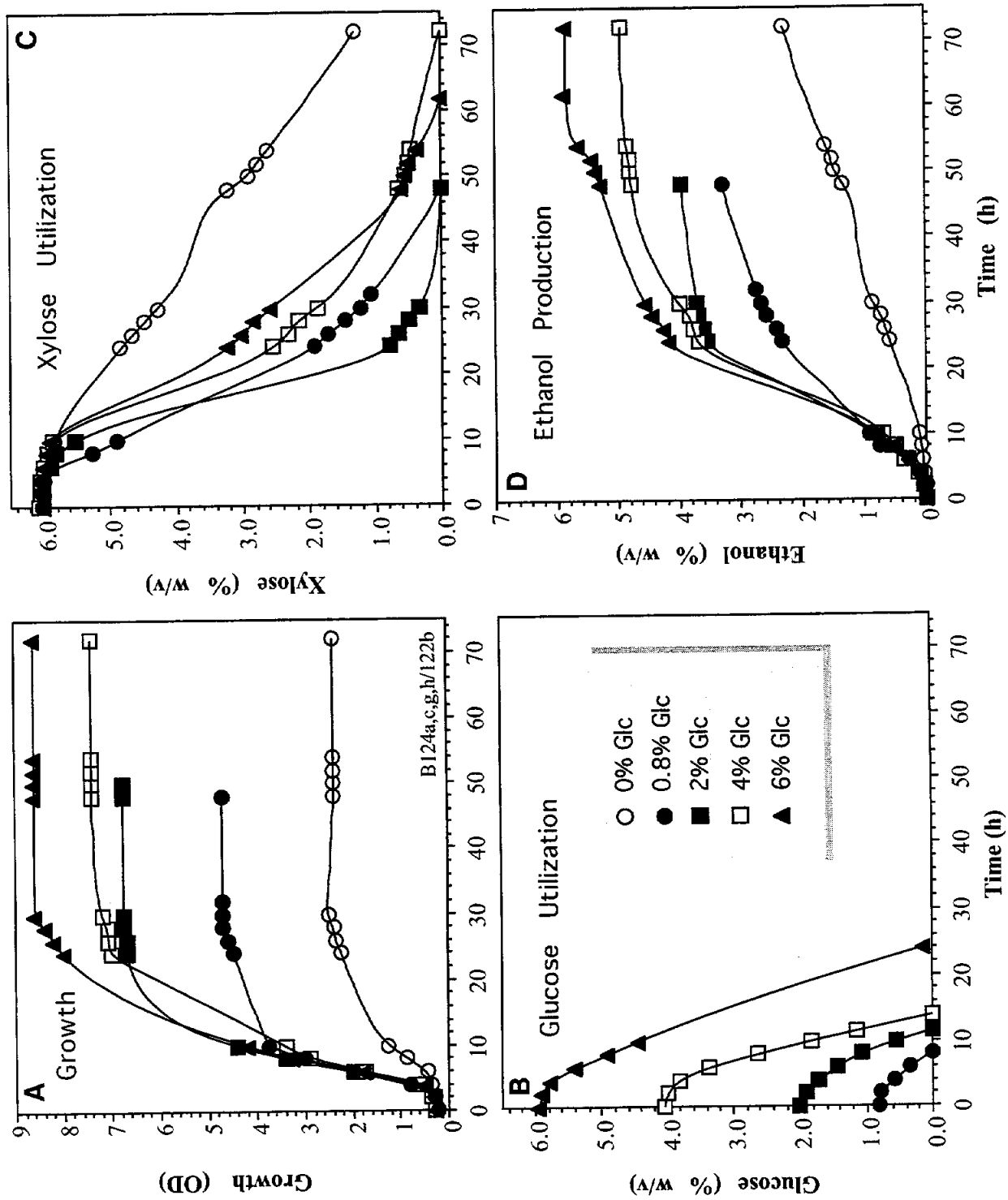

A
Growth
B124a,c,g,h/122b
Growth (OD)
B
Glucose Utilization
0% Glc
0.8% Glc
2% Glc
4% Glc
6% Glc
Glucose (% w/v)
Time (h)
C
Xylose Utilization
Xylose (% w/v)
D
Ethanol Production
Ethanol (% w/v)
Time (h)

FIG 2

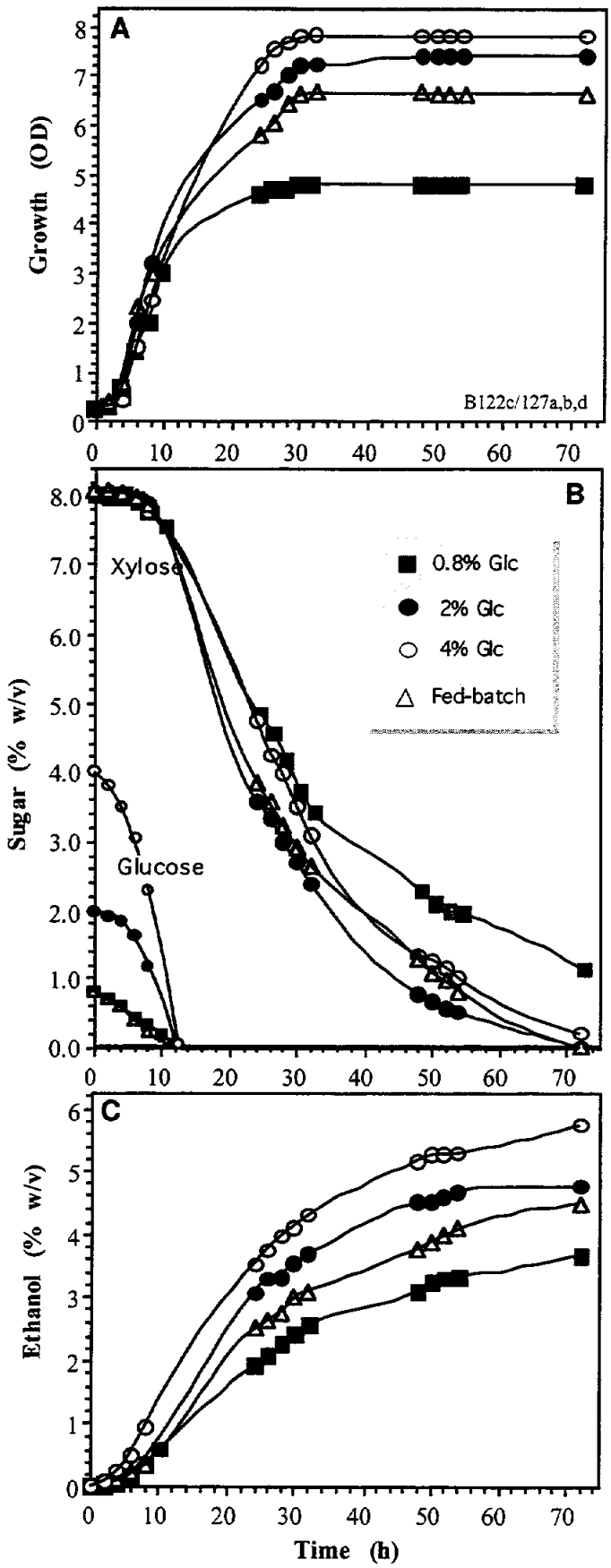

A
Growth (OD)
B122c/127a,b,d
B
Xylose
Glucose
0.8% Glc
2% Glc
4% Glc
Fed-batch
Sugar (% w/v)
C
Ethanol (% w/v)
Time (h)

FIGURE 3

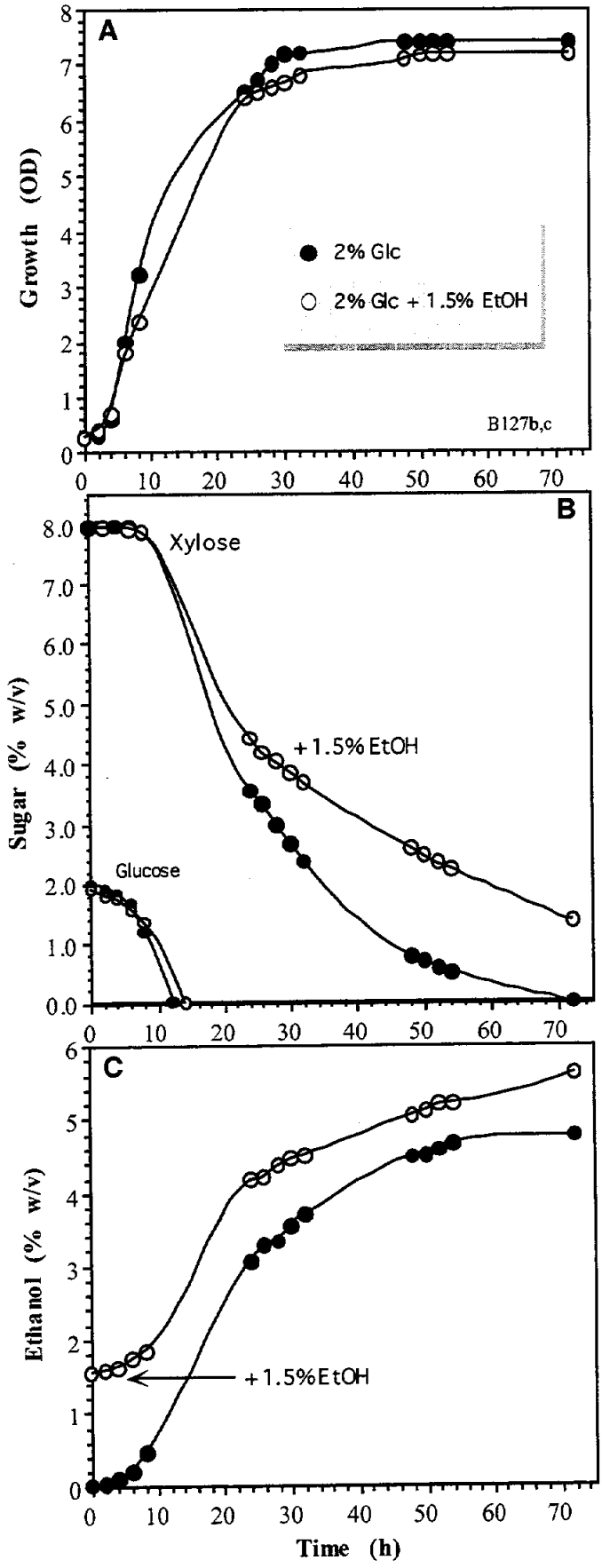
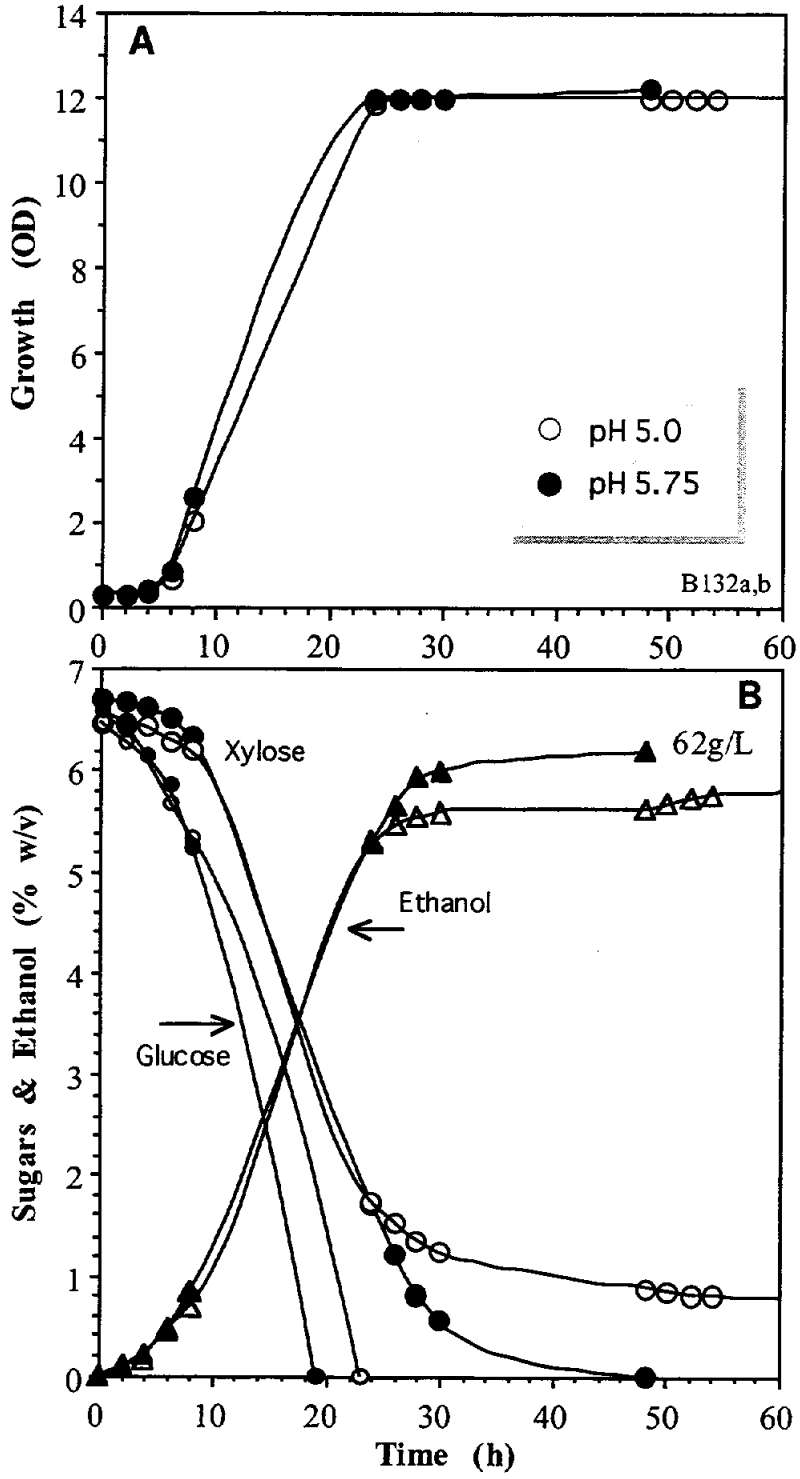

A
Growth (OD)
2% Glc
2% Glc + 1.5% EtOH
B127b,c
B
Xylose
+1.5% EtOH
Glucose
Sugar (% w/v)
C
Ethanol (% w/v)
+1.5%EtOH
Time (h)

FIGURE 4

FIGURE 5


A
Growth (OD)
pH 5.0
pH 5.75
B132a,b
B
Xylose
62g/L
Ethanol
Glucose
Sugars & Ethanol (% w/v)
Time (h)

APPENDIX L

Fermentation performance characteristics of a prehydrolyzate-adapted xylose-fermenting recombinant *Zymomonas* in batch and continuous fermentations

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Fermentation Performance Characteristics of a Prehydrolyzate-Adapted Xylose-Fermenting Recombinant *Zymomonas* in Batch and Continuous Fermentations

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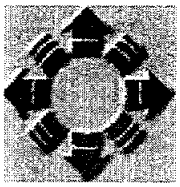

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ABSTRACT

Long-term (149 days) continuous fermentation was used to adapt a xylose-fermenting recombinant *Zymomonas mobilis,* strain 39676:pZB4L, to conditioned (overlimed) dilute-acid yellow poplar hemicellulose hydrolyzate ("prehydrolyzate"). An "adapted" variant was isolated from a chemostat operating at a dilution rate of 0.03/h with a 50% (v/v) prehydrolyzate, corn steep liquor and sugar-supplemented medium, at pH 5.75. The level of xylose and glucose in the medium was kept constant at 4% (w/v) and 0.8% (w/v), respectively. These sugar concentrations reflect the composition of the undiluted hardwood prehydrolyzate. The level of conditioned hardwood prehydrolyzate added to the medium was increased in 5% increments starting at a level of 10%. At the upper level of 50% prehydrolyzate, the acetic acid concentration was about 0.75% (w/v). The adapted variant exhibited improved xylose fermentation performance in a pure sugar synthetic hardwood prehydrolyzate medium containing 4% xylose (w/v), 0.8% (w/v) glucose and acetic acid in the range 0.4-1.0% (w/v). The ethanol yield was 0.48-0.50g/g; equivalent to a sugar-to-ethanol conversion efficiency of 94-96% of theoretical maximum. The maximum growth yield and maintenance energy coefficients were 0.033 gDCM/g sugars and 0.41 g sugars/g DCM/h, respectively. The results confirm that long-term continuous adaptation is a useful technique for effecting strain improvement with respect to the fermentation of recalcitrant feedstocks.

KEY WORDS - recombinant *Zymomonas*, continuous cofermentation, xylose, hardwood prehydrolyzate, ethanol yield, adaptation, acetic acid

Running Title: ***Prehydrolyzate-Adapted Rec Zm***

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INTRODUCTION

The efficient fermentation of xylose-rich hemicellulose hydrolyzates ("prehydrolyzates") represents an opportunity to significantly improve the economics of large-scale fuel ethanol production from lignocellulosic feedstocks (1). Although biomass-derived fuel ethanol is not yet economically competitive with gasoline, significant technological advances in both process configuration and biocatalyst performance have resulted in a progressive reduction in estimated production costs (2,3). It has been proposed that significant additional processing cost reductions can be achieved through a combination of process consolidation (4), economies of scale (3) and improved energy utilization (4,5). For example, advances in bioconversion process consolidation would involve reducing the number of bioreactors through a progression from processes based on sequential hydrolysis and fermentation to ones employing simultaneous hydrolysis and fermentation (6,7). Similarly, advanced designs would employ continuous rather than batch operation. Continuous flow systems configured for cell retention or cell recycling offer the potential of increased productivity and reduced capital expense due to bioreactor size reduction (8).

One of several biomass-to-ethanol processes currently under investigation by the National Renewable Energy Laboratory (NREL) is a simultaneous saccharification and cofermentation (SSCF) process that involves a dilute-acid pretreatment and a patented (9), metabolically engineered *Zymomonas mobilis* that can coferment glucose and xylose (10-14). Encouraging cofermentation performance data with respect to both ethanol yield and productivity have been obtained for recombinant *Zymomonas* using synthetic prehydrolyzate media and pH-stat batch fermentors (15-17). Dilute acid-catalyzed pretreatment is efficient and cost-effect (18-20), but the resulting "prehydrolyzate" contains acetic acid which is known to be inhibitory to ethanologenic microorganisms (2,21,22). The effect of acetic acid on wild-type *Zymomonas* has been documented (23-25). We have studied the effect of acetic acid on xylose utilization by recombinant *Zymomonas* in pH-stat batch (15) and glucose fed-batch fermentations (16). The successful results of preliminary batch trials with recombinant *Z. mobilis* in a laboratory SSCF system using 3.5% (w/v) xylose, 6% (w/v) Sigmacell cellulose and cellulase (25 FPU/g cellulose) were presented at the seventeenth Symposium in 1995 (12). At this year's Symposium, NREL is reporting on pre-pilot scale SSCF trials with recombinant *Z. mobilis* using both pure sugars and a conditioned yellow poplar dilute-acid prehydrolyzate (26).

At this meeting last year we demonstrated the cofermentation performance capabilities of recombinant *Zymomonas* 39676:pZB4L in long-term continuous fermentations using both a synthetic pure sugar medium and a conditioned yellow poplar dilute-acid prehydrolyzate medium (27). Because of the presence of toxic byproducts that are produced during dilute acid-catalyzed hemicellulose hydrolysis, even conditioned prehydrolyzates tend to be recalcitrant to fermentation. In the work that we reported last year (27), the selective pressure of the continuous fermentor yielded an "adapted" variant of 39676:pZB4L, which is the subject of the present investigation. The objective of this work is to describe the physiological characteristics of the prehydrolyzate-adapted recombinant strain in side-by-

side comparisons with the non-adapted culture in both batch and continuous fermentations. Hence, this paper represents a sequel to, or a continuation of, the work that was presented at the nineteenth Symposium last year, and to avoid repetition, this paper relies heavily on the background information regarding experimental rationale, as well as specific methodologies, that have been described previously (27).

MATERIALS AND METHODS

Microorganism *Zymomonas mobilis* 39676:pZB4L (*Z. mobilis* host strain ATCC 39676 transformed with a derivative of the pZB5 plasmid conferring xylose assimilation and fermentation capability, as reported by Zhang *et al.* (10). Cryovials of frozen concentrated stock culture were maintained in RM medium (10 g/L yeast extract and 2g/KH2PO4) supplemented 10 mg/L tetracycline and 15% w/w glycerol at -70°C. Pre-cultures were prepared as described previously (15).

Preparation of inoculum Overnight flask pre-cultures were harvested by centrifugation (16,300 x g for 10min) and the cell pellet resuspended in RM medium without sugar (15) to yield a concentrated cell suspension that was used to inoculate the batch fermentors. The initial optical density (OD, 1cm light path at 600nm) was in the range 0.2-0.25 corresponding to 60-75 mg dry cell mass (DCM) per liter.

Fermentation media The nutrient-rich pure sugar synthetic prehydrolyzate medium used previously (16) was modified to contain the following ingredients per liter of glass distilled water: 40g xylose; 8 g glucose; 5g Difco Yeast Extract (YE) (Difco Laboratories, Detroit, MI); 3.48g KH_2PO_4; 0.8g NH_4Cl; 0.5g $MgSO_4$; 0.01g $FeSO_4.7H_2O$; 0.21g citric acid; 20mg tetracycline. Alternatively, a less expensive pure sugar synthetic prehydrolyzate medium was made with 1% (v/v) centrifugally clarified corn steep liquor (cCSL)(GPC International, Muscatine, IO) as a nutrient substitute for YE. Production of the dilute-acid hardwood prehydrolyzate in a pilot-scale Sunds hydrolyzer, "conditioning" of the prehydrolyzate by overliming, and the preparation of the prehydrolyzate-containing fermentation medium were as described previously (27). Stock pure sugar solutions were sterilized separately.

Batch and continuous fermentation equipment

Batch fermentations were conducted with about 1500ml medium in 2L bioreactors (model F2000

MultiGen, New Brunswick Scientific, Edison, NJ) fitted with agitation (100 RPM), pH, and temperature control (30°C). Continuous fermentations were performed in 750ml NBS C30 chemostats (27) or alternatively with NBS Bioflo 2000 fermentors (1500ml working volume). The pH was monitored using a sterilisable combination pH electrode (Ingold). The standard pH control set-point was 5.75 and the pH was kept constant by the automatic titration with 4N KOH.

Analytical procedures, growth and fermentation parameters

Growth was measured turbidometrically at 600nm (1 cm light path) (Unicam spectrophotometer, model SP1800). In all cases the blank cuvette contained distilled water. Dry cell mass (DCM) was determined by microfiltration of an aliquot of culture followed by washing and drying of the filter to constant weight under an infrared heat lamp. Fermentation media and cell-free spent media were compositionally analysed by HPLC as described previously (15). The ethanol yield ($Y_{p/s}$) was calculated as the mass of ethanol produced per mass of sugar consumed. The volumetric ethanol productivity was determined by dividing the final ethanol concentration by the total batch fermentation time. Bracketed values for volumetric productivity indicate that sugar utilization was incomplete when the experiment was terminated. For chemostat cultures, the maximum mass growth yield (ie. corrected for maintenance) ($_{max}Y_{x/s}$) was determined as the inverse of the slope in a plot of the specific sugar utilization rate (q_s, g sugar/gDCM/h) as a function of the dilution rate (D, 1/h) where the y-axis intercept represents the value for the maintenance energy coefficient (m_e, g sugar/gDCM/h).

RESULTS and DISCUSSION

Generating the "adapted" variant of rec Zm 39676:pZB4L This study focuses on the fermentation performance of the xylose-utilizing recombinant *Z. mobilis* in a pH-controlled continuous flow bioreactor (chemostat) using both synthetic and real hardwood prehydrolyzate. Our objective was to use the selective pressure provided by the continuous growth environment of the chemostat to achieve strain improvement by the "adaptation" resulting from the long-term exposure of the recombinant to incremental increases in the amount of prehydrolyzate in the feed medium. The selective pressure exerted on an organism within the controlled growth environment of the continuous flow bioreactor (chemostat) is a powerful research tool for effecting strain improvement through a process of acclimation or adaptation that takes place during the long-term exposure to gradually

increasing levels of an inhibitory substance(s).

In the work presented last year on continuous culture studies with rec Zm 39676:pZB4L (27), we described the initial stages of a long-term chemostat experiment that was intended to generate a prehydrolyzate-adapted variant of rec Zm 39676:pZB4L. At the beginning of the experiment the CSL-based medium contained 10% (v/v) dilute acid-catalyzed yellow poplar prehydrolyzate (with pure sugar supplementation to achieve glucose and xylose levels of 0.8% w/v and 4% w/v, respectively). These sugar concentrations were selected because they were similar to the concentrations in full strength hydrolyzate liquors obtained by dilute acid pretreatment of hardwood (yellow poplar) sawdust (28). Batch fermentations had shown that 30% hydrolyzate significantly inhibited fermentation performance of the recombinant; however, at the 10% level, performance was not inhibited (29)

The chemostat culture experiment shown in Figure 1 is a continuation of the experiment illustrated in Figure 5 of our previous study (27) in which the chemostat was operated at a dilution rate of 0.04/h for 55 days. Over this period of time the prehydrolyzate concentration had been increased incrementally from 10% to 35% (v/v) where the final acetic acid concentration was about 0.5% (w/v). This level of acetic acid is reasonably inhibitory to batch fermentation performance (15). In this study the continuous culture was continued at a relatively constant dilution rate of 0.03/h for another 94 days and over this period the prehydrolyzate level of the medium was increased from 35 to 50% (v/v) in 5% (v/v) increments (Fig. 1). The acetic acid concentration increased from 0.5% to about 0.75% (w/v) (Fig. 1). After 94 days of continuous operation the effluent xylose and ethanol concentrations were 0.68 and 2.1% (w/v), respectively (Fig. 1). Over the entire time course of the experiment, glucose was seldom detected in the chemostat effluent (results not shown). This level of ethanol represents a process ethanol yield (based on sugars available) of 0.44g/g (conversion efficiency of 87% theoretical). The culture that was isolated at the termination of this long-term (149 days) experiment was designated as the "adapted" variant of rec Zm 39676:pZB4L. This hardwood prehydrolyzate-adapted strain is the focus of the present comparative physiological assessment.

Comparison of cofermentation performance adapted and non-adapted recombinants

Figure 2 compares the growth and cofermentation performance of rec Zm 39676:pZB4L and adapted variant in batch fermentation using a pure sugar nutrient-rich synthetic prehydrolyzate medium with

the pH controlled at 5.75. The mineral salts and yeast extract-based ("ZM") medium contained 4% (w/v) xylose and 0.8% (w/v) glucose, but no acetic acid. Under this condition, the performance exhibited by the two strains was remarkably similar (Fig. 2). Although the adapted strain produced a lower final culture turbidity (Fig. 2A), the final cell mass concentrations were similar (Table 1). One possible distinguishing feature of the adapted strain revealed in Fig. 2B was the apparent slower rate of glucose utilization. A separate experiment with glucose as the sole sugar confirmed that an idiosyncrasy of the adapted strain is a slower growth with, and metabolism of, glucose, as well as a lower growth yield (Fig. 3). In comparing the time-courses of the batch fermentations shown in Figures 2 and 3, it is important to note the difference in scale of the x-axes. Under these assay conditions, any difference between the two strains was not expected since the medium did not contain any inhibitory substances (principally acetic acid) to which the adapted strain might have become less sensitive.

Comparative cofermentation performance in acetic acid-containing media

Since the adapted strain was isolated from a chemostat that was operating with a feed containing 50% (v/v) prehydrolyzate (0.75% w/v acetic acid), it seemed reasonable to assume that altered sensitivity to acetic acid inhibition might be one way to characterise the adapted strain. In a previous study we documented the acetic acid sensitivity of rec Zm 39676:pZB4L and showed that 0.4% acetic acid caused a 50% inhibition of growth and cofermentation (16). Using a CSL-based synthetic prehydrolyzate medium containing 0.4% (w/v) acetic acid, the adapted and non-adapted recombinants were compared in pH-stat batch fermentations where the pH was controlled at 5.0, 5.5 or 6.0 (Fig. 4). At all three pH values the adapted strain outperformed the non-adapted recombinant with respect to both growth and xylose fermentation; however, the ethanol yield remained close to theoretical maximum for both strains independent of the pH (Fig. 4, Table 1). The highest ethanol productivity (0.61g/L/h) was achieved by the adapted strain at pH 6 (Fig. 4, Table 1).

Figure 5 compares the growth and fermentation performance at pH 6 using the same medium but with the acetic acid concentration increased to 1% (w/v). At this relatively high level of acetic acid, the adapted strain achieved a slightly higher cell mass concentration (Fig. 5A); however, the rate of xylose utilization was significantly faster with the adapted strain relative to the non-adapted strain (Fig. 5B). For both strains the ethanol yield (based on sugar consumed) was 0.48g/g (conversion efficiency of 94% theoretical maximum) (Table 1). In the context of rec Zm strain specificity with

respect to acetic acid sensitivity, it is interesting to note that our previous work with rec Zm CP4:pZB5 (15) indicates that it possibly rivals the adapted strain in terms of resistance to acetic inhibition.

Characterisation of continuous cofermentation with "adapted" recombinant

Figure 6 shows the steady-state concentrations of xylose, ethanol and cell mass as a function of dilution rate for a chemostat culture of the adapted recombinant using a CSL-based pure sugar synthetic prehydrolyzate medium. The results shown in Figure 6 are very similar to those observed under the same conditions using the non-adapted recombinant. The similar pattern observed in continuous cofermentation was expected both from the similar performance behaviour observed in batch cofermentation and the fact that the medium did not contain any potentially inhibitory substances. In the context of continuous cofermentations with recombinant *Zymomonas*, we noted that in a recent report on performance assessment of rec Zm CP4:pZB5, Rogers *et al.* (30) stated "in chemostat culture for 40g/L glucose plus 40g/L xylose medium, that full sugar utilization occurred in the dilution rate range 0.05-0.06/h with ethanol concentrations close to 40g/L" (p305). This observation by Rogers *et al.* (30) is of particular interest since recombinant CP4:pZB5 exhibits more tolerance to acetic acid (15) than rec Zm 39676:pZB4L (16).

Figure 7 shows a Pirt (31) plot of q_s and q_p versus D for the chemostat culture with the adapted recombinant. Regression analysis of the specific sugar utilization rate data gives values of 0.033 g/g and 0.41 g/g-h for $^{max}Y_{x/s}$ and m_e, respectively (Fig. 7). These values compare to values of 0.042 g/g and 1.13 g/g-h observed previously, under the same conditions, using the non adapted recombinant (27). As well as being influenced by several environmental factors, these physiological and bioenergetic parameters are known to be strain specific (27). "Apart from the recent work of Joachimsthal *et al.* (25) that showed an increase in m_e with increasing amounts of acetate, the literature is silent on the subject of the effect of acetic acid on the maintenance energy coefficient in *Zymomonas*." In this context it is interesting to note that the m_e value for an acetate-tolerant ZM4 mutant was determined to be 1.9 g/g-h at pH 5.4 with about 0.9% (w/v) acetic acid (25). This can be compared to an average value for m_e of 1.6 g/g-h for strain ZM4 in the absence of acetate (32). The lower maintenance coefficient exhibited by the adapted recombinant is interesting and may provide a clue regarding the mechanism by which this variant is able to tolerate higher levels of acetic acid since this substance is known to act as an energetic uncoupler (23). Clearly, the mechanism by which the

adapted recombinant achieves an improved fermentation performance in the presence of acetic acid is an area for further research.

In a separate study being presented at this meeting, the adapted strain has been used in integrated bench-scale SSCF experiments with yellow poplar prehydrolyzate and lignocellulosic solids to total solids loadings of 14% w/v (26). The combined results of these two studies certainly auger well for the proposed further testing with this biocatalyst at pilot scale. This study provides support for the contention that long term continuous culture is an effective technique for effecting strain improvement (27).

Acknowledgments

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Figure Captions

Fig. 1 Adaptation of recombinant *Z. mobilis* 39676:pZB4L to overlimed yellow poplar prehydrolyzate. Experimental details are given in *Materials & Methods*. This represents a continuation of the chemostat experiment described in Figure 5 of ref #27.

Fig. 2 Comparative performance of rec Zm 39676:pZB4L and adapted variant in pH-stat batch fermentation with a pure sugar nutrient-rich synthetic prehydrolyzate medium (**A**) Growth, and (**B**) sugar utilization and ethanol production. The ZM medium contained 4% (w/v) xylose and 0.8% (w/v) glucose (no acetic acid); the pH was 5.75 and the temperature was 30°C. The values for maximum dry cell mass concentration, ethanol yield and productivity are given in Table 1

Fig. 3 Comparative performance of rec Zm 39676:pZB4L and adapted variant with glucose as sole sugar source (**A**) Growth, and (**B**) glucose utilization and ethanol production. The ZM medium contained 4.8% (w/v) glucose (no acetic acid); the pH was 5.75 and the temperature was 30°C. The values for maximum dry cell mass concentration are shown in panal A

Fig. 4 Effect of 0.4% (w/v) acetic acid on rec Zm 39676:pZB4L and adapted variant in batch fermentations as a function of pH (**A**) Growth, pH 5, (**B**) sugar utilization and ethanol production, pH 5, (**C**) growth, pH 5.5, (**D**) sugar utilization and ethanol production, pH 5.5, (**E**) growth, pH 6, (**F**) sugar utilization and ethanol production, pH 6. Symbols: O, non-adapted rec Zm; ●, adapted recombinant. The mineral salts medium contained 1% (v/v) clarified CSL with 4% (w/v) xylose and 0.8% (w/v) glucose (*Materials & Methods*). The values for maximum dry cell mass concentration, ethanol yield and productivity are given in Table 1

Fig. 5 Effect of 1% (w/v) acetic acid on rec Zm 39676:pZB4L and adapted variant in a CSL-based pure sugar synthetic prehydrolyzate medium. (**A**) Growth, (**B**) sugar utilization and ethanol production. The medium was the same as described in Fig. 3. The pH was 6 and the temperature was 30°C. The values for maximum dry cell mass concentration, ethanol yield and productivity are given in Table 1

Fig. 6 Steady-state concentrations of xylose, ethanol and cell mass as a function of dilution rate for pure sugar continuous cofermentation using "adapted" recombinant *Z. mobilis*: The medium was the same as described in Fig. 2. There was no acetic acid in the medium and no glucose was detected in the chemostat effluent. The pH was 5.75 and the temp. was 30°C.

Fig. 7 Specific rates of sugar utilization and ethanol production by recombinant *Z. mobilis* as a function of dilution rate. See Fig. 6 for description of experimental conditions.

Table 1 Summary of growth and fermentation parameters

pH	Acetic acid % (w/v)	Maximum Cell Mass (g DCM/L)	Maximum Ethanol (g/L)	Ethanol Yield (g/g)	Ethanol Productivity (g EtOH/L/h)
Non-adapted recombinant					
5.75*	0	1.38	23.6	0.48	0.79
5.0	0.4	0.73	24.0	0.49	(0.35)
5.5	0.4	0.96	24.9	0.50	0.44
6.0	0.4	1.06	24.2	0.49	0.48
6.0	1.0	0.63	15.8	0.48	(0.22)
"adapted" recombinant					
5.75*	0	1.34	23.6	0.48	0.90
5.0	0.4	0.88	24.8	0.50	0.51
5.5	0.4	1.04	24.2	0.50	0.55
6.0	0.4	1.18	24.3	0.49	0.61
6.0	1.0	0.72	21.2	0.48	(0.29)

* The medium was "ZM" with 4% (w/v) xylose and 0.8% (w/v) glucose. All other fermentations were with 1% (v/v) clarified CSL-based media (see Materials & Methods) with the same sugar concentrations.

Brackets around values for Ethanol Productivity indicate that xylose utilization was incomplete when batch fermentation was terminated

Figure 1

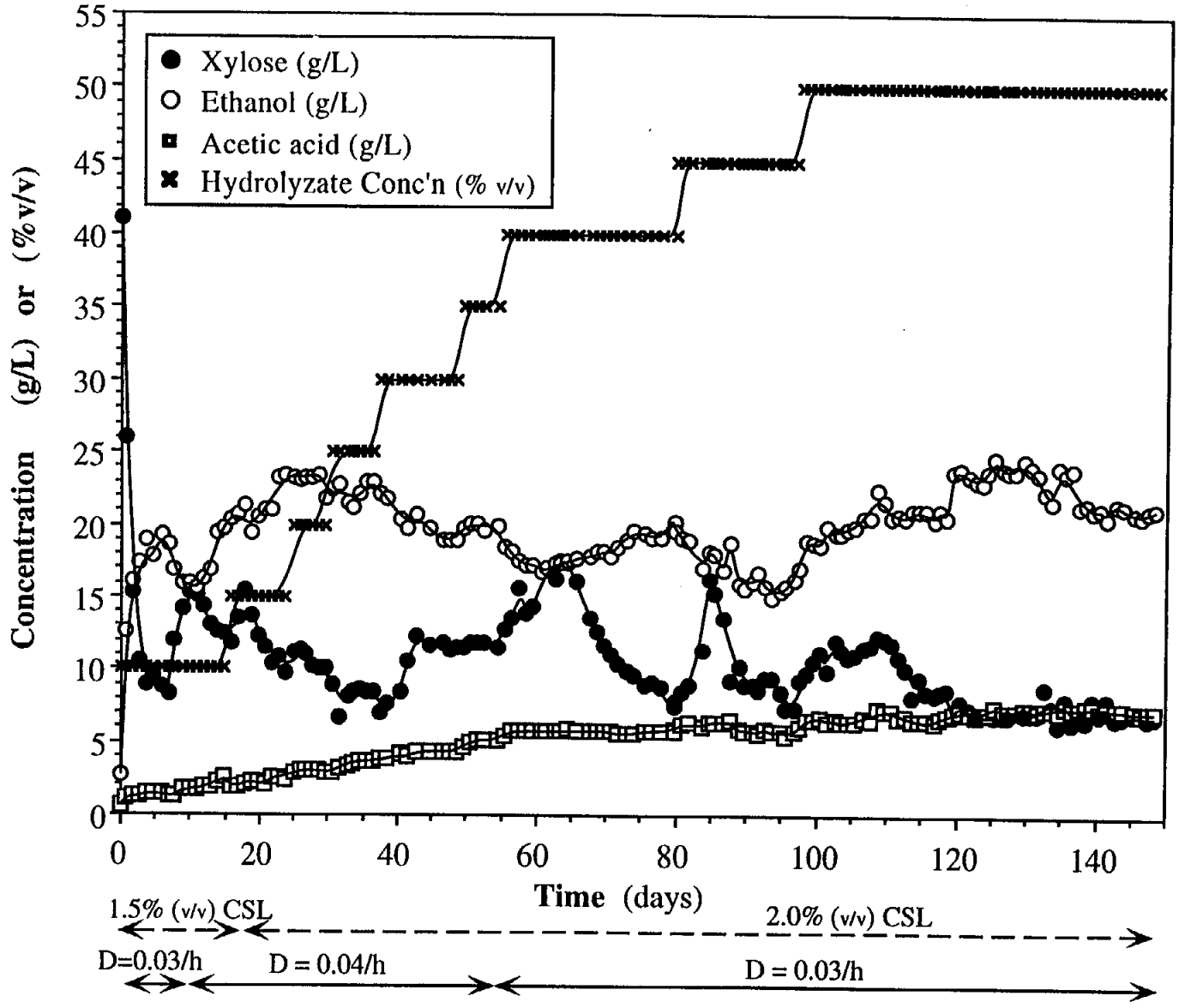

Xylose (g/L)
Ethanol (g/L)
Acetic acid (g/L)
Hydrolyzate Conc'n (% v/v)
Concentration (g/L) or (%v/v)
Time (days)
1.5% (v/v) CSL
2.0% (v/v) CSL
D=0.03/h
D = 0.04/h
D = 0.03/h

Figure 2

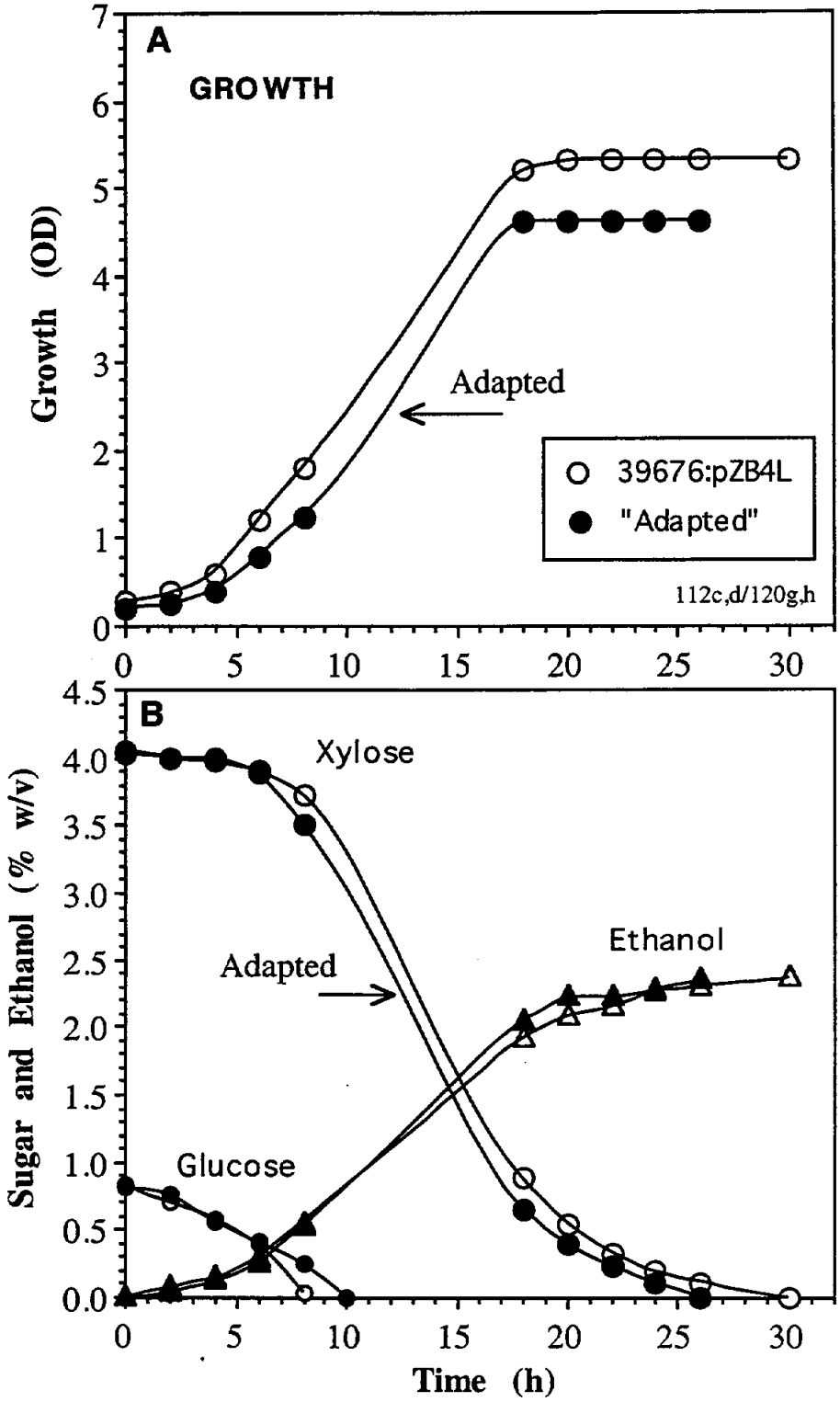
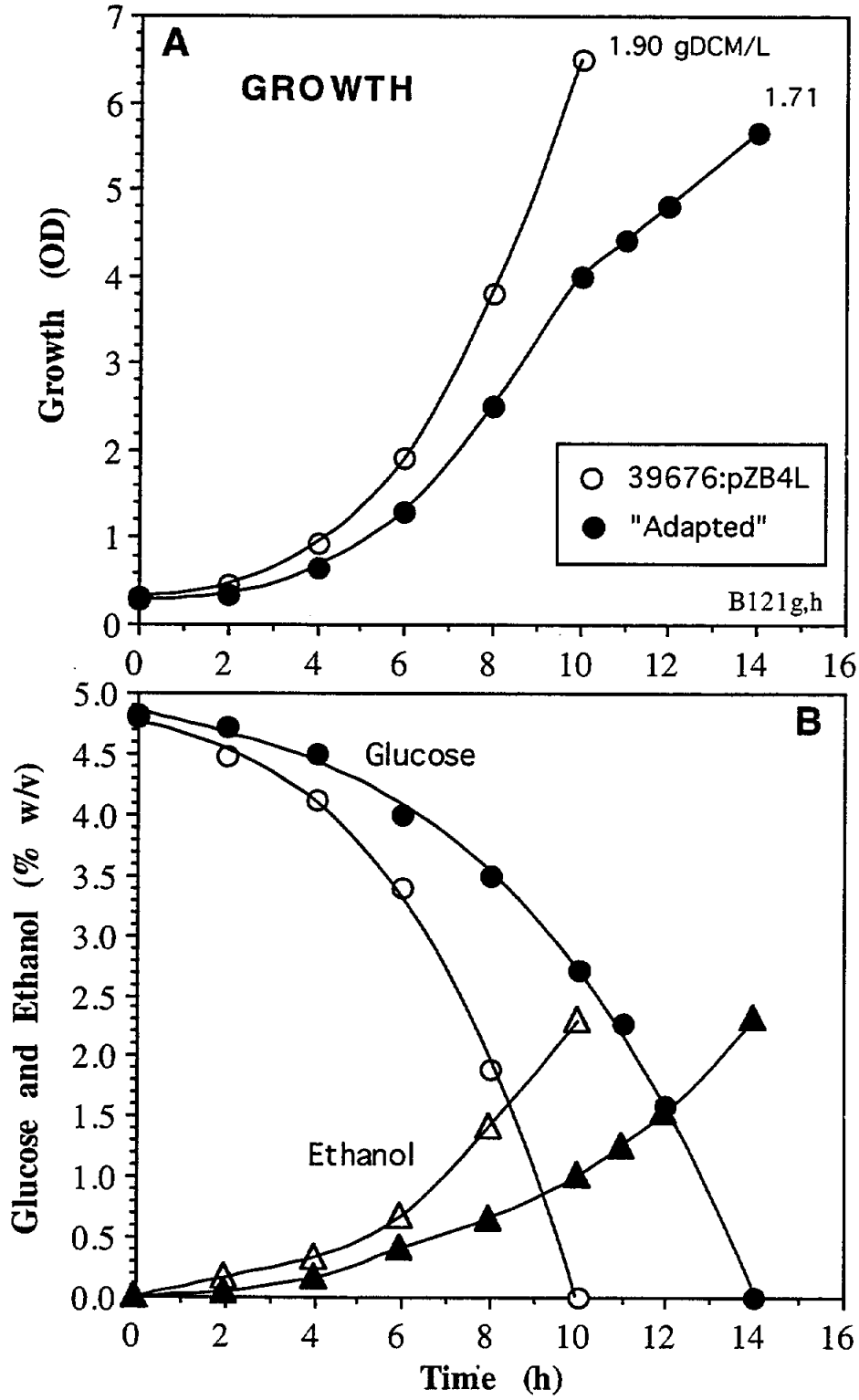

A
GROWTH
Growth (OD)
Adapted
39676:pZB4L
"Adapted"
112c,d/120g,h
B
Xylose
Adapted
Ethanol
Glucose
Sugar and Ethanol (% w/v)
Time (h)

Figure 3


A
GROWTH
1.90 gDCM/L
1.71
Growth (OD)
39676:pZB4L
"Adapted"
B121g,h
B
Glucose
Ethanol
Glucose and Ethanol (% w/v)
Time (h)

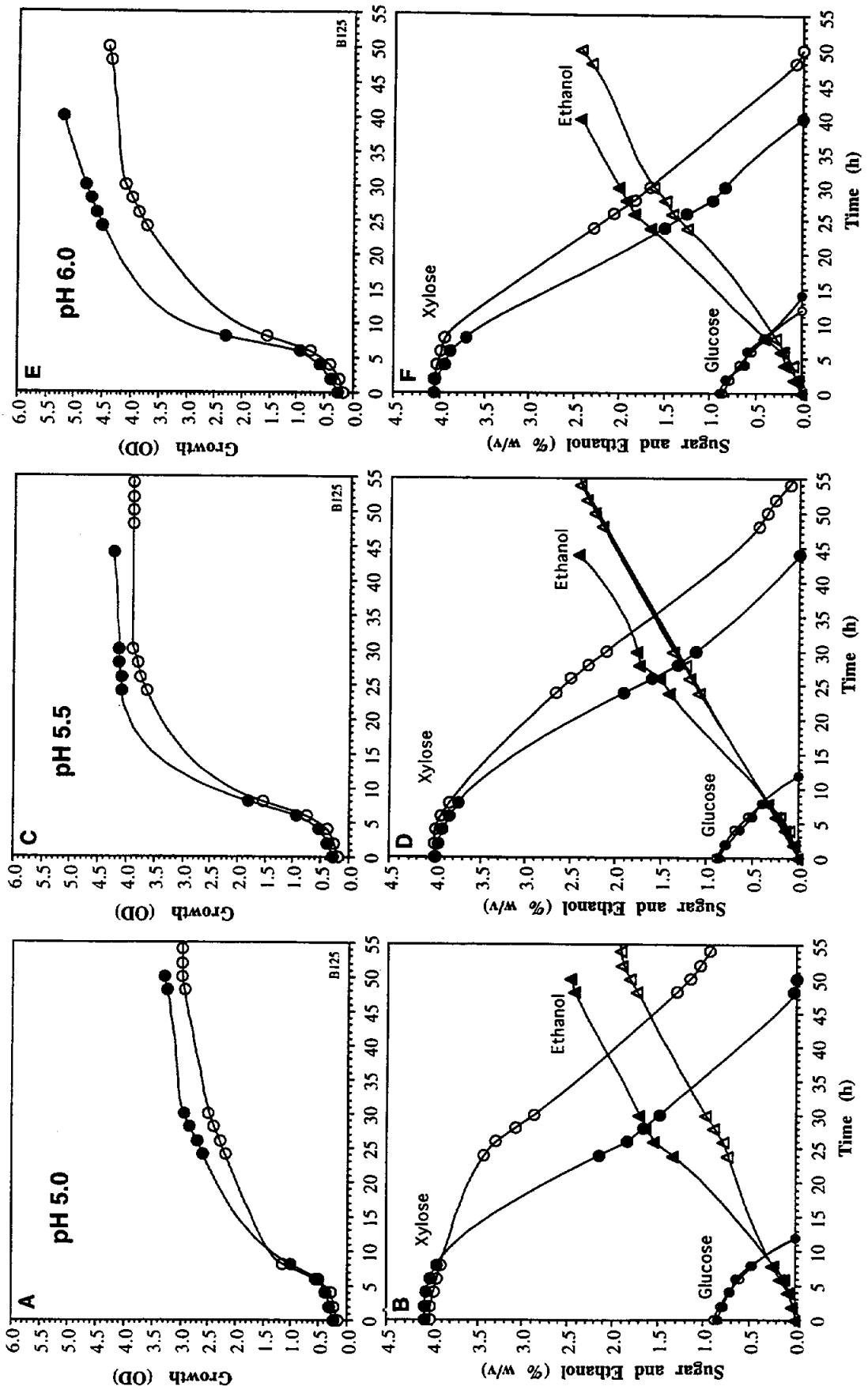

A
pH 5.0
B
C
pH 5.5
D
E
pH 6.0
F
B125
Growth (OD)
Sugar and Ethanol (% w/v)
Xylose
Glucose
Ethanol
Time (h)

Figure 4

Figure 5

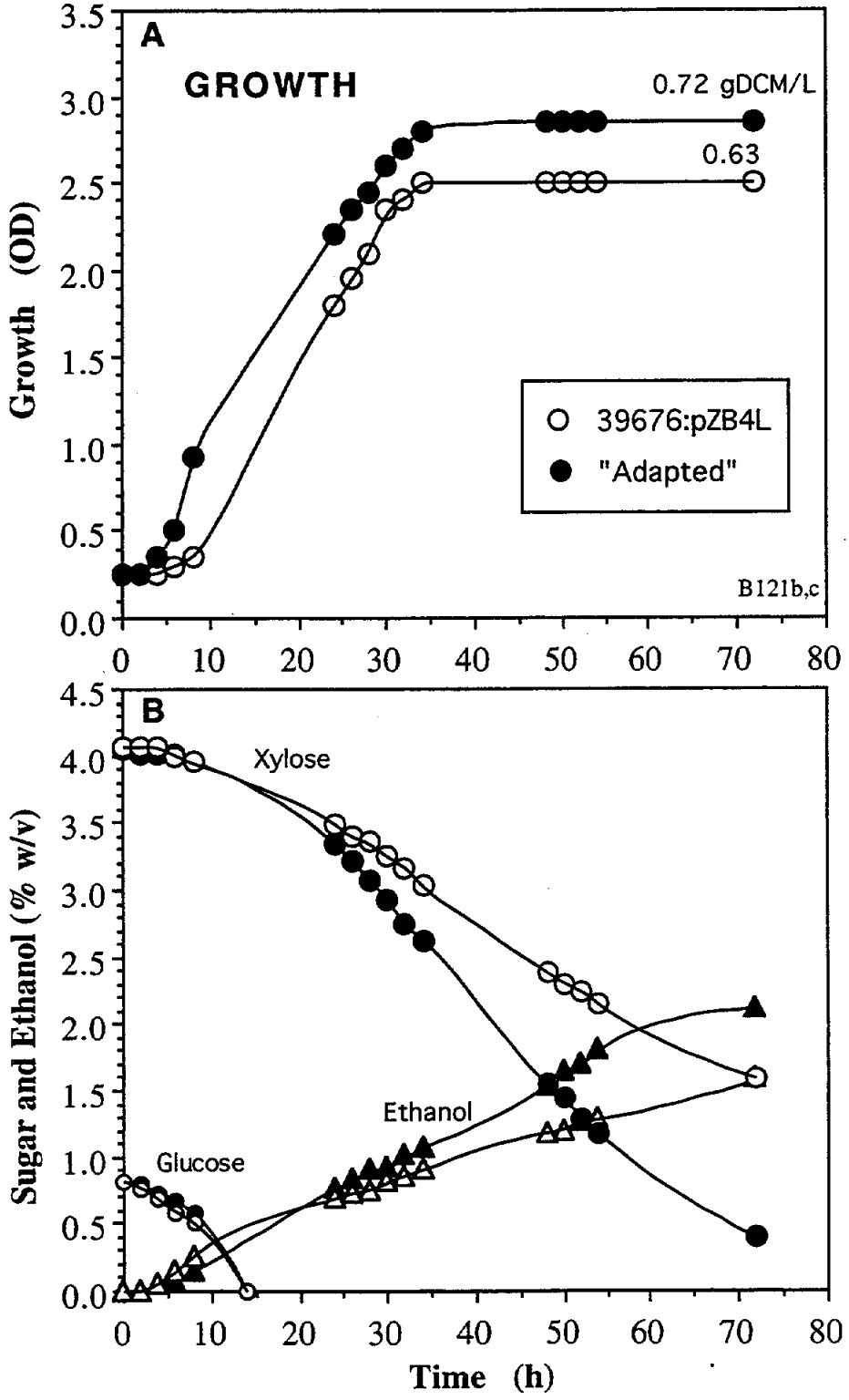

A
GROWTH
0.72 gDCM/L
0.63
39676:pZB4L
"Adapted"
B121b,c
Growth (OD)
B
Xylose
Ethanol
Glucose
Sugar and Ethanol (% w/v)
Time (h)

Figure 6

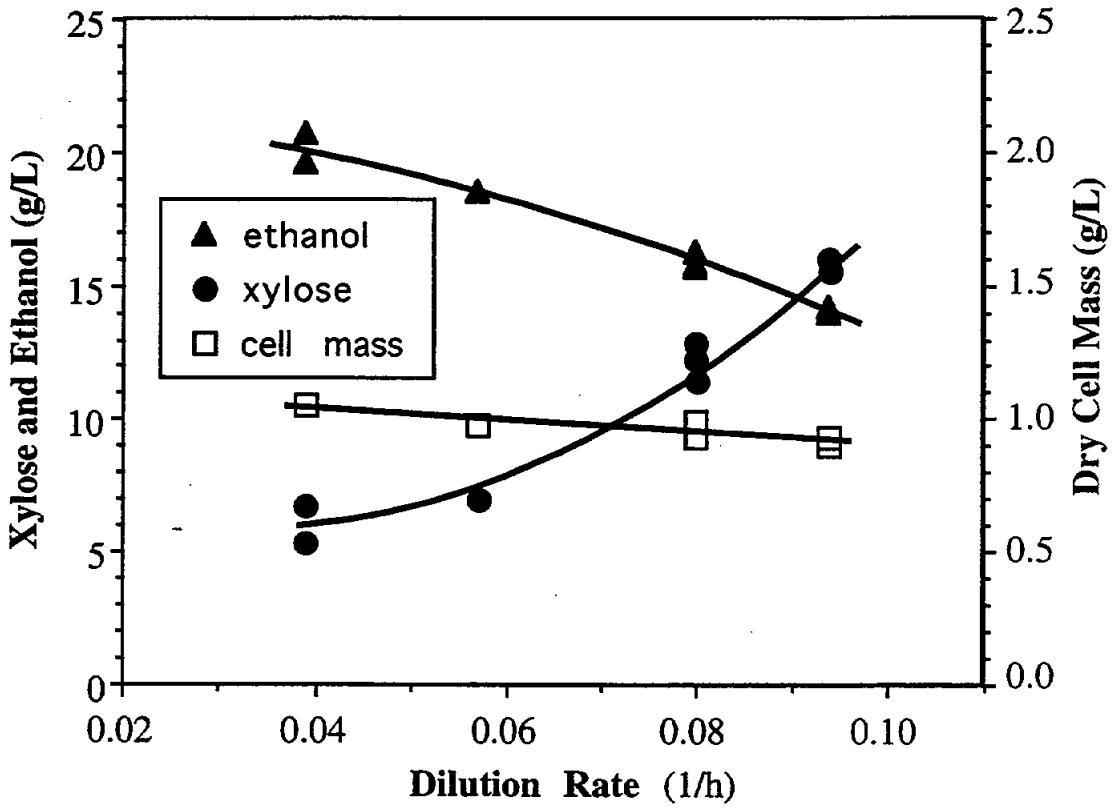
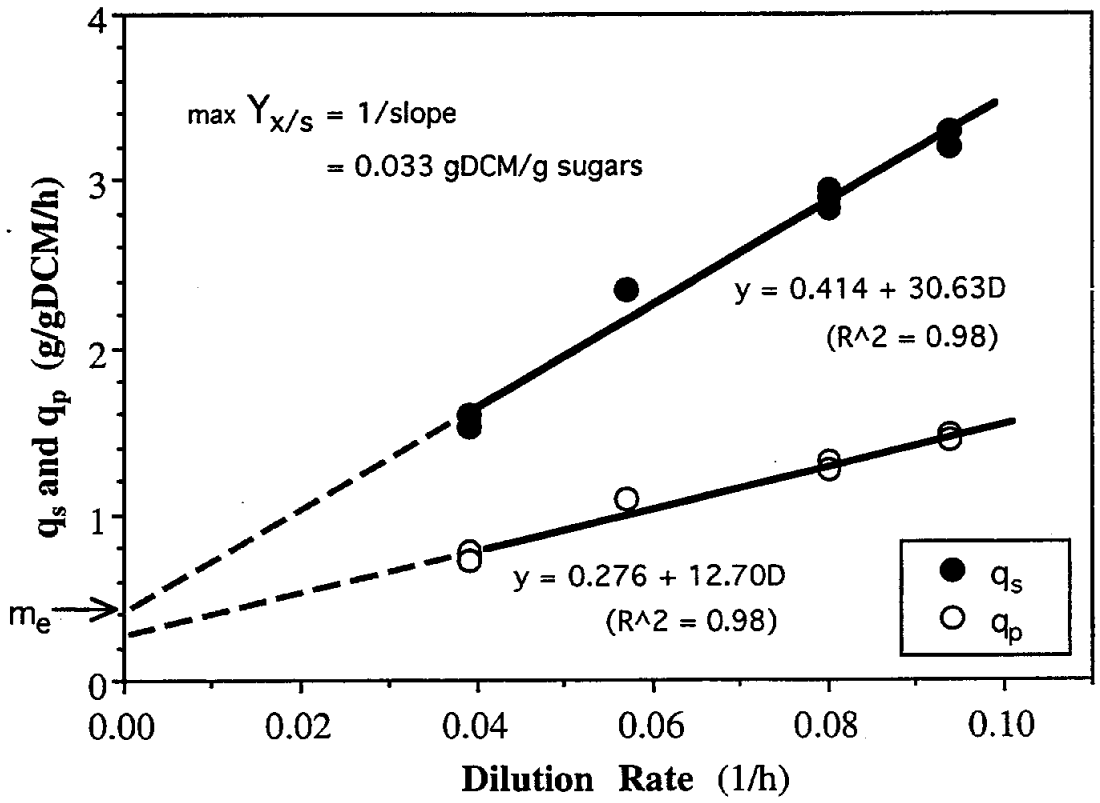

25
20
15
10
5
0
2.5
2.0
1.5
1.0
0.5
0.0
0.02
0.04
0.06
0.08
0.10
ethanol
xylose
cell mass
Xylose and Ethanol (g/L)
Dry Cell Mass (g/L)
Dilution Rate (1/h)

Figure 7


max $Y_{X/S}$ = 1/slope
= 0.033 gDCM/g sugars
y = 0.414 + 30.63D
(R^2 = 0.98)
y = 0.276 + 12.70D
(R^2 = 0.98)
q_s
q_p
m_e
q_s and q_p (g/gDCM/h)
Dilution Rate (1/h)
0
1
2
3
4
0.00
0.02
0.04
0.06
0.08
0.10